A DISCUSSION ON THE PART PLAYED BY THE SUPRAVESTIBULAR CONNECTIONS IN DECEREBRATE RIGIDITY

By Dr L J J Muskens (*Amsterdam*)

During the past few years I have had but limited laboratory facilities for performing experimental operations connected with the remarkable phenomenon of decerebrate rigidity, but I have succeeded in carrying out a number of experimental lesions in cats involving the region of the posterior commissure

The cases here recorded are only those where the animal survived for a sufficiently long period to make the subsequent examination of the nervous tissue with the osmic acid stain practical.

I consider it important to distinguish those cases which present complete immobility with opisthotonos as described by Sherrington, Horsley and Loewenthal from those with increased tonicity on one side, the power of locomotion being preserved (1)

It is not surprising to anyone who has made a study of the effect of lesions in the posterior longitudinal fascicle and commissural nuclei to find forced movements closely associated with decerebrate rigidity Further, because symmetrical lesions are very rare in this region phenomena related to forced movements are bound to occur Sherrington has all along described conditions, either of stimulation or of extirpation which alter the condition of decerebrate rigidity or give it a special character

The original writers on the subject denied the existence of cerebellar influences on the phenomenon of decerebrate rigidity, but it must be admitted that later work has demonstrated that the anterior cerebellar lobe does exert some influence Further, different writers are not in agreement in their descriptions of the effect of lesion and stimulation of the crus anterior cerebelli and Gowers' tract The fact, however, seems to be established that ablation of Deiters' nucleus, hemisection of the medulla oblongata, section of the antero-lateral tract of the cord and also of the posterior roots does away with the rigidity when it has once appeared

Thiele, Weed, Warner and Olmsted demonstrated that unilateral section of the truncus cerebri and the rhinencephalon produced a certain

degree of rigidity, certainly at least a temporary lateral increase of muscle tone It is curious to note that if this unilateral section is made above (*i e* anterior to) the posterior commissure the resulting stiffness is heterolateral, while if the section be caudal to the commissure the stiffness is homolateral

In the first observations on decerebrate rigidity it was noticed that a special posture was associated with the condition, the neck was bent, the concavity being directed backwards Sherrington recognised in a complete demonstration of decerebrate rigidity a "physiological entity" Such a posture is a forced position in the vertical plane such as I described as occurring after certain lesions involving the primary and secondary vestibular connections(2) Although Sherrington speaks of a special posture Magnus speaks of a "caricature of a posture," because the physiological movement upward is exaggerated If, however, the dictum of Liddell and Sherrington is recalled which states "Reflex standing is a composite postural reflex the anti-gravity muscles counteracting the superincumbent weight,"(3) it is justifiable to expect *a priori* that the mesencephalic structures which govern these anti-gravity muscles may play an important part when the interruption of certain inhibiting tracts sets the action of these structures free

Here it is worth while to recall a forgotten observation of Ewald to the effect that a pigeon blindfolded and with both labyrinths removed takes the extreme position of "culbutation" backwards, neck and eyes turned maximally overhead Again certain observations in the pigeon tend to prove, that upward movement of the eyes is dependent on an entirely different mechanism, compared with lateral and downward movement, and might be considered as an effect of release from various innervations Also it is well known, that in man during sleep the eyeballs are directed upwards

The following points appear to me to require consideration Warner and Olmsted concluded that the spino cerebellar and Gowers' tracts supplied the efferent proprioceptive impulses for the phenomenon of decerebrate rigidity, hence stimulation of these tracts would alter the fundamental conditions of the phenomenon

It must be recalled that Marburg and Bing showed that lesions of these tracts were followed by forced movements in the vertical plane and a tendency to fall backwards On the other hand, Warner and Olmsted concluded that the efferent impulses necessary for decerebrate rigidity arose in the neighbourhood of the red nuclei but not in the red nuclei themselves

In birds, although a true condition of decerebrate rigidity could not be induced, Martin Rich observed quite accurately in birds that forced movements in the horizontal plane on one side associated with a tendency to fall to the other side invariably followed after cross-section of the mid-brain.

Weed noted that hæmorrhage in the raphe of the truncus cerebri may cause flexion decerebrate rigidity instead of the exaggerated posture of the extensors

Magnus and de Kleijn have shown that the condition of decerebrate rigidity varied considerably with the position of the animal at the time

Decerebrate rigidity has always been regarded as a release phenomenon ("Enthemmung") in which severance of the prosencephalon from the mesencephalon was held to be the essential feature Although this was the general belief yet it was realised that the proof was unsatisfactory

Ken Kure recently propounded the view that the occurrence of hæmorrhage was one of the conditions necessary for the exhibition of decerebrate rigidity From my own observations it seemed to me very probable that some special alteration in the condition of the mid-brain such as hæmorrhage would induce is necessary for the demonstration of complete decerebrate rigidity On the other hand, the results of observations by Bazett, Penfield and Rademaker seem to exclude the possibility of such a complication being a main factor in bringing about the condition

In the thirty years which have elapsed since decerebrate rigidity was first recognised two main lines of investigation have been followed. In the first and earlier work attention was directed to the different influences affecting decerebrate rigidity In the later work, started by Weed and completed by Rademaker, attention was concentrated on the part played by the red nucleus in the occurrence of this phenomenon. It is remarkable that the second and later group of workers dealt with the subject primarily as physiologists the anatomical considerations coming second The examination of all records of the work done on the subject shows no careful analysis of the forced movements in three planes while no direct application of the latest anatomical knowledge concerning the supravestibular nuclei is attempted

This curious incompleteness and one sidedness of the investigation in my opinion accounts for the doubt and uncertainty found in all the literature dealing with decerebrate rigidity

Before discussing the effect of lesions of the commissural nuclei and their efferent tracts, or attempting to answer the question as to the rôle played by a lesion involving the ascending and descending tertiary vestibular connections in the occurrence of decerebrate rigidity, certain anatomical-physiological data must be recalled As a result of the work of Cajal, who first suggested the possibility, it was definitely established in 1907 that vestibular nerve fibres pass right into the nucleus tecti cerebelli without any cell stations This was confirmed later by Villaverde and W F Allen(4) Wallenberg and Deganells observed in birds, vestibular fibres passing to the lateral nucleus, whereas Trendlenburg, Marx and Frenhel contradicted this statement Since this date (1907) I have repeatedly directed attention to the fact that a lesion of this roof-nucleus always brings about forced movements in the vertical plane, characterised especially by a tendency to fall backwards with the head and eyes directed upwards In birds culbutation backwards is peculiarly well marked Occasionally in the first few days after the lesion has been performed, when compensation is not yet effective, forced movement in the opposite direction is seen, the animal falling forward or displaying forward culbutation In the case of forced movement in the vertical plane backwards and culbutation backwards I noticed an important point of difference from contrary-wise directed forced movements in the vertical plane—falling forward or culbutation forward—and also from the other types of forced movements in the horizontal and frontal planes (these later types of forced movement I have already dealt with in earlier papers, *loc cit*) It is this, forced movement backwards is always associated with an increase in muscle tone, especially of the extensors This may be due to the fact that the direction of the movement is against gravity while the forced movement in the opposite or forward direction is with gravity Further, forced movements in the forward direction appear to be associated with a curious tendency to sleep

It must be clearly realised that the centres and muscles involved in these two forms of forced movements in the vertical plane are not and could not be mirrors (Spiegelbild) one of the other, as is the case with circus movements and rolling movements In the case of circus movements the locomotion towards the right is really the complete "mirror" of the movement towards the left In the case of rolling movements to either side the same holds true

In discussing the cause of the increased muscle tone found in forced movements backwards, whether these movements are brought about by

a lesion of the fastigial nucleus (nucleus tecti) or of its hypothalamic connections[1], it must be recalled that in a series of experimental hemisections a temporary stiffness was found heterolaterally if the lesion were anterior to the posterior commissure or homolaterally if posterior to it Further Graham Brown found that when the cross-section of the mid-brain of a monkey is stimulated electrically in front of the posterior commissure, conjugate deviation of the head and eyes to the unaffected side resulted. The earlier work of Knoll and Topolanski, on the other hand, proved that such stimulation applied behind the posterior commissure produced conjugate deviation of head and eyes to the affected side Further it is known that in a series of hemisections of the brain stem from the region of Deiters' nucleus upwards the forced movements in the horizontal and frontal planes change in direction as soon as the posterior commissure is reached(2)

Is it possible with the available data to approach the subject of decerebrate rigidity and discuss it profitably?

In such a discussion certain errors made by earlier workers can at least be avoided. For example, the fact that the red nucleus and the bundle of Monakow, both prominent neural entities, showed marked degeneration in cases of decerebrate rigidity, led to the conclusion that the red nucleus must be the principal factor in this phenomenon. Not only does the case I shall proceed to describe oppose such a view but the work of Bazett and Penfield and Thiele also oppose such a conclusion. It must at the same time be admitted that as yet no cases of genuine decerebrate rigidity have been examined where the bundle of Monakow has entirely escaped degeneration. Why such a case is unlikely to occur has been suggested earlier in this paper Finally in reptilians where the red nucleus is but very slightly developed, cross-section of the mid-brain

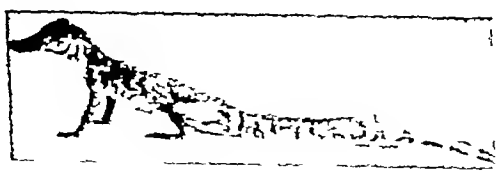


Fig 1

[1] F Bremer *Arch internat de Physiol* 19 p 192 1922. These researches have gone far to remove any doubt as to the presence of increased muscle tone after lesion of the fastigial nucleus. The possibility that this phenomenon of increased muscle tone is not so much associated with any particular centre as with the movement itself evidently did not occur to the writer

produces a peculiar condition(5) very comparable with decerebrate rigidity in higher mammals (Fig 1)(6)

From a general consideration of the evidence it is obvious that attention must be concentrated on the work of von Gehuchten, Cajal, de Lange and Castaldi who agreed in the view that a set of nuclei exist about the posterior commissure which have been proved to be of peculiar significance in relation to forced movements, and which are now recognised to be relay centres for the ascending secondary vestibular connections (refer to the work of Vogt(7) and Riese(8)) So far as is at present known about these nuclei it would appear that they are connected with a large number of ascending vestibular fibres but give rise to a very much smaller number of descending fibres These descending fibres are situated in the innermost part of the posterior longitudinal bundle and are probably possessed of a functional significance far superior to their anatomical size As is known, even the most primitive type of vertebrates possess these cells and fibres and in some a particular gigantic nerve fibre—Mauthnerz's fibre—does possess a very important function in relation to locomotion Van Gehuchten originally concluded from observations made in fishes and on the embryonic stages in higher animals, that the whole of the posterior longitudinal bundle was composed of such descending fibres Further it is known that the ascending and descending fibres of this bundle are the first to be provided with a medullary sheath

Rademaker of the Utrecht school has all along been one of the most zealous supporters of the theory that the red nucleus does play an important part in decerebrate rigidity His statement that a mid ventral incision in the base of the mid brain 2½ mm in depth does not induce decerebrate rigidity, while a similar incision 3½ mm in depth does is very important. At the same time I am inclined to consider the conclusion drawn by Rademaker not completely justified. It is a well known anatomical fact that the nucleus interstitialis lies in front of the anterior pole of the red nucleus but these two nuclei are not separate and distinct entities but merge one into the other It is therefore impossible to localise a lesion explicitly to the red nucleus in the way Rademaker suggests Further, unless the animal has lived six or seven days after such an experiment as that performed by Rademaker, it is impossible to ascertain by the osmic acid method whether the red nucleus has or has not escaped. One of his own cases (called "Black and white) might be brought forward to support an argument that both the red nucleus and nucleus interstitialis play a part in decerebrate rigidity, but the objection does exist that this animal did not live long enough after the operation, and the rigidity was not classical in type Further, in Rademaker's experiments the preliminary operation alone produced as a rule forced movements, this in itself rather invalidates conclusions drawn from the second operation This is more important because at the site of the preliminary operation the secondary vestibular connections finish and the tertiary begin, so that each lesion has in all probability a profound effect on both secondary and tertiary structures. The experiments described by Rademaker, where traction was made, on a thread fixed medially are interesting, but unfortunately

the subject of experiment (*A A*) was killed directly and no microscopic examination of the brain tissue was made. In this case it can be supposed that the supra vestibular connections associated with vertical forced movement escaped. In the other two cases ("Grisette" and 'Kleine Zwarte') Meynert's decussation was certainly severed while there was no degeneration of Monakow's bundle, but the cases were not typical examples of decerebrate degeneration. In one case (Grisette) the tr. interstitio spinalis or Boyce's tract was degenerated. This tract has curiously enough been called the tractus dorsalis. In two other cases (B T and B P) a permanent posture in the form of rotation round the axis existed, yet in these cases the correcting reflexes of the labyrinth were present. This encourages me to think that my criterion for a possible lesion in the posterior longitudinal bundle (tendency to forced movement) is a better one, and gives a more exact test than the presence or absence of the righting reflexes. Where Rademaker criticises my experiments and maintains that other lesions than those affecting the posterior longitudinal bundle may cause forced movements, he merely adduces the fact that hemianoptic animals may deviate in the horizontal plane. This is sometimes the case with pigeons but it passes quickly and it is not valid to compare a pigeon with cats and rabbits, the animals used in my research(9). Munzer and Wiener have shown the preponderance of optic impressions and the number of optic nerve fibres in pigeons greatly exceeds the number of all the other sensory fibres.

Further, in Rademaker's recent work on the anatomical exploration of the mid brain the sharp differentiation of the different forms of forced movement is inadequately dealt with. In animals which survived for a sufficiently long period care was not taken to prepare sections stained by the Marchi method for demonstration purposes, neither were the microscopic preparations so prepared as to indicate clearly the left and right sides[1]. The same objections apply to the stimulation experiments of Graham Brown(10). In this case the red nucleus was stimulated and the effect on an existing condition of decerebrate rigidity noted. Here again the proximity of commissural nuclei excludes the possibility of a definite decision.

Langworthy studied the condition of decerebrate rigidity in very young animals as well as in some of the lower types. He conceives of the art of walking as the result of two component factors. One is postural, a contraction of the extensor muscles overcoming the force of gravity and enabling the animal to stand, the other is progressive, and is achieved by rhythmic flexion and extension of the legs. Although the most important component—the act of falling forward—is omitted, yet an important contribution is made to the subject. In the young opossum cross section of the mesencephalon is followed first by prominent progressive movements which later in the life of the animal are replaced by a typical condition of decerebrate rigidity. It is interesting to find that Langworthy calls the forced movement backwards which follows cross-section of the secondary (crus anterior cerebelli) and tertiary (resection of both nuclei caudati) vestibular connections an accentuation of the postural reflex'.

From a general consideration of the whole problem it seems impossible to hold any profitable discussion on the phenomenon of decerebrate rigidity without a true appreciation of the tracts and structures associated with the forced movements, more especially those in the vertical plane. For example, how fallacious would the conclusions be drawn from

[1] In order to distinguish the right and left sides in microscopical preparations it has been my custom for many years to make a small incision with a razor on the right side of the basal surface of the brain stem and cord as soon as the hardening with Müller's fluid has taken place, to make perfectly certain the horse hair used to keep the brain sections apart pierces the sections only on the right side of the median line.

mesencephalic experiments if no account were taken of the known facts concerning the direction of circus and rolling movements The case described by Spiegel and Nikishawa (11) illustrates this point Case 9 was an animal where the posterior longitudinal bundle had been severed on the left side The animal performed, quite in accordance with my observations, circus movements to the right The authors now state that hemisection in front of the red nucleus causes increased unilateral tonicity of the muscles of the trunk and fore paws and that this induces circus movements to the right Such apparently contradictory results are resolved when the effect of lesions of the posterior longitudinal bundle are considered.

To establish the rôle played by the commissural supra-vestibular nuclei in the causation of decerebrate rigidity attention must be drawn to the following points

1 Complete decerebrate rigidity ensues only if vestibular connections, lesion of which causes vertical forced movements, are severed on both sides

In my case, No 242, which exhibited classical decerebrate rigidity, there was no lesion of either pyramidal tracts After this experiment it is superfluous to admit influence from the cerebral hemispheres[1]

2 The complete reversal of the physiological effect at the level of the posterior commissure must not be forgotten This is well demonstrated when a series of oral to caudal hemisections are made across the brain stem of a quadruped and the condition and localisation of partial and temporary rigidity noted, both on the side of the lesion and on the opposite side

3 This reversal is also seen where in different animals the posterior longitudinal bundle is severed on one side Caudal to the posterior commissure the ensuing circus movements are directed heterolaterally, rolling however to the affected side, while the direction of both is reversed as soon as the posterior commissure is reached (2)

4 Graham Brown found the same complete physiological reversal when oral caudal sections were made through the brain in primates when the area of the posterior longitudinal bundle was stimulated

From a consideration of these data it is evident that section through the secondary and tertiary vestibular connections exerts a very definite influence on the occurrence of decerebrate rigidity Especially a certain influence must be admitted by those tracts and structures, injury of which results in forced movements in the vertical plane A certain influence may be ascribed to the red nucleus, but as yet proof of this assumption is not forthcoming

1 The animal survived the lesion five days There were no signs of degeneration in the pyramidal fibres, either ascending or descending, neither were the pedunculi cerebri injured.

With all deference to the work of Luciani, Horsley and Clark and many others, very little is as yet clearly understood about cerebellar localisation and co-ordination. It is known that even a superficial lesion of the cortex diminishes the faradic irritability of the contralateral motor cortex (Rossi(12), Bikeles, Muskens(13))

Further, it is known that decerebrate rigidity is inhibited by stimulation of the palæo-cerebellum It may even be admitted that the cerebellum has at least an influence on tonic-postural phenomena (Rossi and Simonelli(14)) In further study of the nature of cerebellar influence it will be well to distinguish the essentially vestibular function of the fastigial nuclei from the purely cerebellar function of the dentate nucleus The cortex of the mid-lobe might conceivably serve as a relay station for both To analyse the influence of the brachium anterior cerebelli, and the cerebellum as a whole on decerebrate rigidity four different factors have to be recognised. These are

(1) Gowers' and Flechsig's spino-cerebellar tracts carrying proprioceptive impulses to those parts of the cerebellum (palæo-cerebellum) which when faradised produce increased muscle tone (Horsley, MacNalty, Bremer)

(2) A secondary vestibular bundle leaving the nucleus fastigii and passing probably partly through and below the lateral wing of the posterior longitudinal bundle to reach the nucleus anterior of the thalamus The exact identity of this bundle and its postulated crossing in the velum is as yet a matter of debate Probst and Luna described this tract as an "accessorische Bindarm," but van Gehuchten, Wallenberg, Allen and others are not convinced of its existence In birds, however, Frenkel(15) has put the existence of the tractus cerebello-diencephalicus as an uncrossed part of the brachium conjunctivum beyond doubt Physiologically speaking some such connection must be postulated, for I have convinced myself that in several cats after cross-section of the brachium conjunctivum, particularly if the lesion be on both sides, forced movements in the vertical plane (staggering, falling backwards opisthotonos, increased tone in extensors or occasionally falling forwards) ensued, just as after a lesion of the fastigial nucleus

(3) The principal cerebellar bundle, i e tractus dentato-rubralis

(4) That part of the brachium conjunctivum that is built up by the descending fibres which originate in the basal longitudinal bundle (Wallenberg)

The case to which I would draw attention is a cat in which on May 3rd, 1914, a lesion was affected on the lamina medullaris lateralis thalami on the right side. A degeneration

in Forel s field followed, probably lesion of the tractus pallido commissuralis This lesion caused very little alteration in the behaviour of the animal except for some deviation in the horizontal plane

In this operation the internal capsule escaped any injury this was proved by the absence of degeneration in the pes pedunculi On May 5th formol was injected in the endeavour to cause degeneration of the nuclei tecti This attempt succeeded so far both nuclei being affected, for the injection entered in the mid line between the two nuclei and degeneration was found in this commissure and both nuclei tecti After this lesion the animal demonstrated backward movement retrograde oulbutation or creeping forward crouched low on the floor In this operation probably secondary vestibular (fastigial) fibres on both sides were interrupted.

It was next (May 18) attempted to cut both basal longitudinal bundles just in front of the posterior commissure by a deep mid ventral incision (Figs 2, 3 and 4)

Fig 2 Section showing most caudal part of mid brain lesion

In this case all the vestibular connections associated with vertical movements were severed. In the earlier operations the lamina medullaris externa on the right side and the commissure between both nuclei tecti cerebelli were injured. The condition of decerebrate rigidity which ensued after the last vertical incision (Figs 2 3 and 4) (the pyramidal tracts being uninjured, the tr rubro spinalis showing no degeneration) was more striking than any demonstration of the phenomenon seen by me either in my own or in Horsley s laboratory or elsewhere

The condition lasted the five days which the animal survived after the last operation but there were some alterations in posture as indicated in Figs 5 *a b c d* If the limbs were moved passively they invariably regained the forced position with a curious spring like movement.

In this case the red nucleus (Monakow s bundles showed no sign of change) and the pyramidal tracts escaped It was, however equally certain that in the final operation the

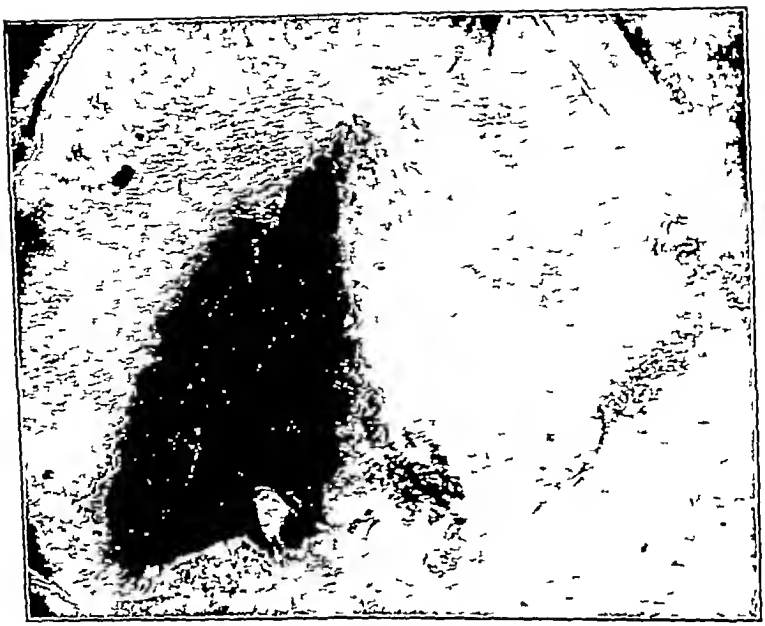
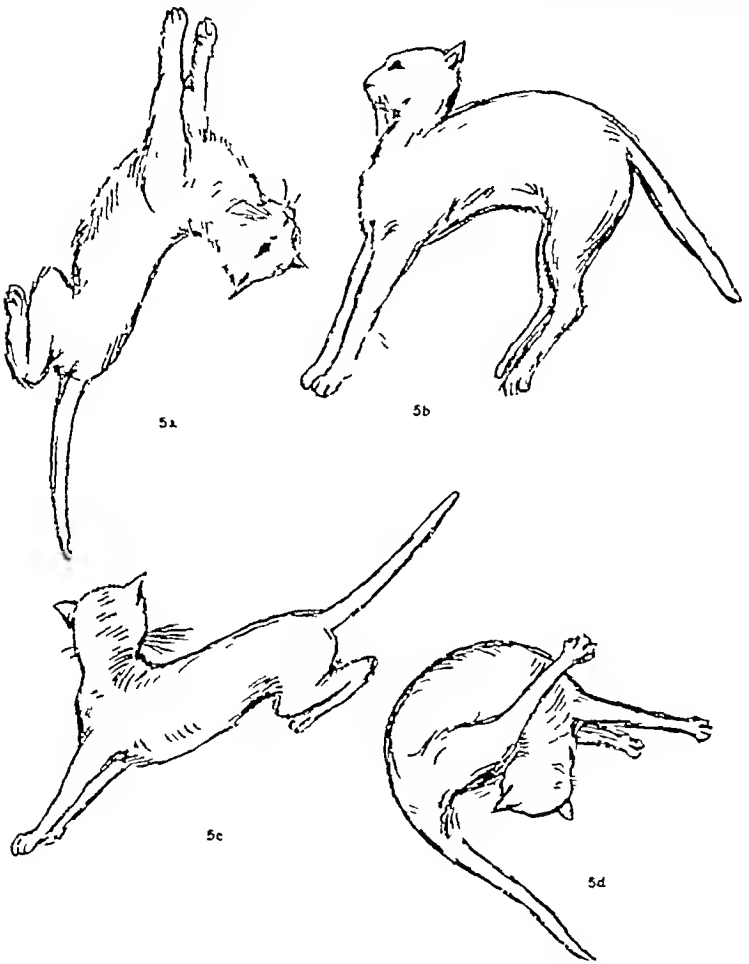


Fig 3 Section showing lesion in its greatest extent.
Reproduced from Muskens' *Epilepsy* by permission of Messrs Baillière, Tindall & Cox

Fig 4 Section showing oral part of lesion.

interstitial nucleus was injured. This is shown in Fig 6 where incipient degeneration in the closely adjacent tractus interstitio spinalis on one side, and on the other side (Fig 7) in



Figs 5*a, b, c, d* Posture in which cat 242 was found on four subsequent days

the fibres connecting the commissural nuclei and the globus pallidus is found (The line of section is directed more anteriorly on the right than on the left side)

The interpretation of this case of decerebrate rigidity appears to me to be as follows. The rigidity reached the supreme degree because the influence of both nuclei tecti was accentuated in the second operation by the median mesencephalic incision which produced a maximal degree of decerebrate rigidity

In this case therefore is an example of a double interruption of the secondary vestibular tracts, dealing with forced movements in the vertical plane and frontal plane, including tr interstitio spinalis

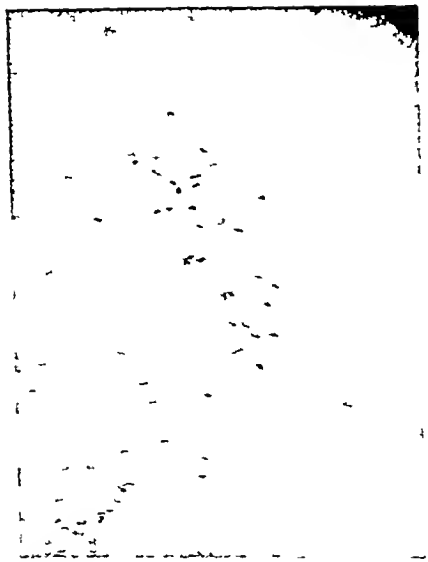


Fig 6 Enlarged. P L.B of Fig 4. Showing incipient degeneration of tr interstitio spinalis.

Fig 7 Enlarged from Fig 4. Fibres ascending from commissural nuclei towards glob pallidus on the right side

With such evidence it must be admitted that the part played by the supra vestibular connections in the production of decerebrate rigidity is very considerable This applies especially to the ascending and descending bundles which are associated with movements in the vertical plane

Sherrington has shown that removal of both labyrinths does not inhibit decerebrate rigidity, and according to Bernis and Spiegel(13) even the destruction of Deiters' nucleus and of the nucleus rami descendens VIII is compatible with the existence of decerebrate rigidity If, however, the anterior spinal column is interrupted decerebrate rigidity breaks down on the affected side In such a case the descending tracts from the commissural nuclei (especially the tractus interstitio spinalis) or Boyce's tract are probably involved

Summary

1 In former publications on decerebrate rigidity the writers have dealt with the subject primarily as physiologists, anatomical considerations coming second

2 Forced movements backwards in the vertical plane, whether caused by a lesion of the primary vestibular centre, by section of the ascending secondary connections, by lesion of the supra-nuclear centre near the posterior commissure, or its palæo-striatal connection are probably always associated with a certain amount of rigidity, recalling Sherrington's "composite postural reflex, the antigravity muscles counteracting the super-incumbent weight"

3 From different observations it is seen that there is a complete reversal of physiological effect at the level of the posterior commissure

(*a*) In a series of oral to caudal hemisections across the brain-stem of a quadruped temporary rigidity, homo- and hetero-lateral, is noted

(*b*) If an incision is made in the region of the posterior longitudinal bundle forced movements in the frontal and horizontal plane are directed reversely, accordingly as the incision is on the caudal or oral side of the commissure

(*c*) The same complete reversal is observed after faradic stimulation of the same region, when oral-caudal sections are made through the brain of primates

4 Since section of the pyramidal tract has probably nothing to do with the origin of decerebrate rigidity, and as the nucleus ruber is frequently found intact, the part played by the supra-vestibular connections in the production of the phenomenon may be supposed to be considerable This applies especially to the ascending and descending bundles, lesion of which is associated with forced movements in the vertical plane

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THE BLOOD-PRESSURE REFLEXES OF THE RABBIT UNDER URETHANE ANÆSTHESIA

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URETHANE is an anæsthetic very frequently used either alone or in combination with other drugs for work on rabbits The following observations were made in the course of some investigations upon the cerebral circulation, with special reference to the reflex from the carotid sinus extensively described by H E Hering(1)

Briefly, according to this author, this reflex is concerned with the regulation of the general blood-pressure level and is subserved by a distinct nerve—the sinus nerve Stimulation of the sinus region or of the nerve results in a fall of blood-pressure

Hering, in his monograph, gives many tracings from rabbits, anæsthetised, at least partially, with urethane It is the purpose of this communication to point out that the effects of this anæsthetic complicate any conclusions drawn from experiments on blood-pressure reflexes in the rabbit

We are indebted to Sir Charles Sherrington for pointing out to us that depressor reflexes in the rabbit can be obtained from numerous procedures after the administration of large doses of chloral This fact directed our attention to the urethane

Intravenous urethane was found to be an excellent anæsthetic for rabbits when recovery, after operation, was desired For this purpose 75 grm. per kilo of 25 p c solution was slowly injected into an ear vein after which anæsthesia occurred very rapidly For operative procedures the urethane was supplemented by ether Recovery was complete from this dose in about 12 hours and without any obvious after effects

For the acute experiments on reactions obtained from the carotid sinus, the urethane was administered in the same way and in the same or slightly larger dosage[1] For the operative procedures ether was also

[1] Urethane has been found to have the same action on blood pressure reflexes when administered intraperitoneally (1 5 grm. per kilo).

given, but was discontinued after the complete setting up of the preparation as it was found that the urethane provided adequate, though light, anæsthesia It was in this condition that the tracings appended were obtained When the effects of stimulation were to be compared with those obtained under ether alone, the depth of anæsthesia was regulated so as to be as nearly as possible the same as under the urethane.

Sollman and Brown (2) were the first to describe the fall of systemic blood-pressure consequent on traction on the peripheral end of the common carotid artery This can be very easily verified in dogs and cats (under ether) and rabbits, and the mechanism has been analysed by Hering It is present in the rabbit even under ether anæsthesia, but the accompanying tracing (Fig 1) demonstrates the remarkable effect of the change

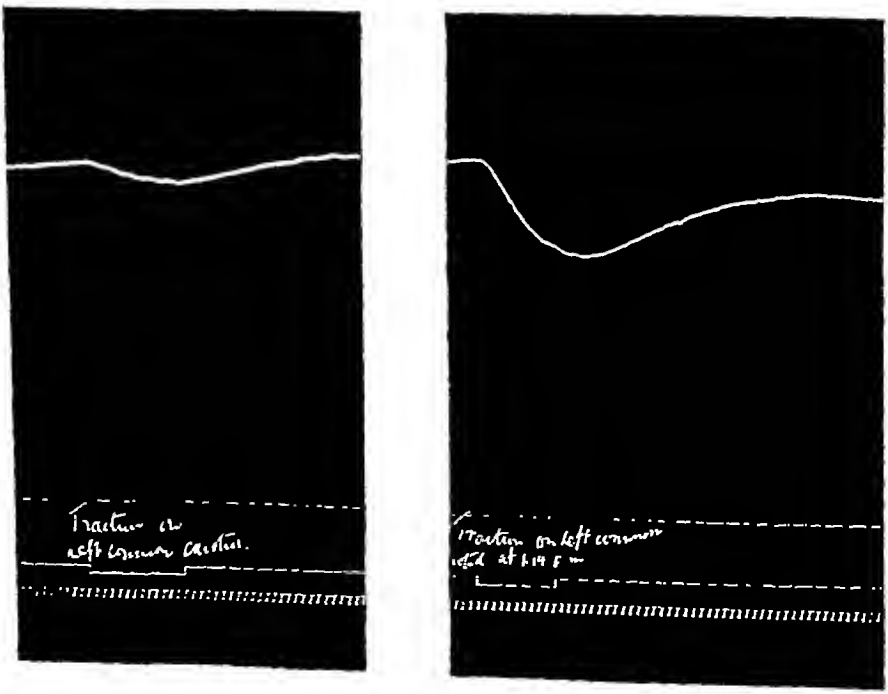


Fig 1 Rabbit Traction exerted on peripheral end of common carotid artery Tracing shows slight fall of blood pressure under ether and the pronounced fall under urethane anæsthesia Time in seconds

from ether to urethane anæsthesia (In all cases the blood-pressure was measured in the femoral artery)

With ether, traction on the peripheral end of the left common carotid artery produces a slight but perceptible fall With urethane there is a very marked fall That is to say, the urethane has "sensitised" the reflex, present to a slight degree under ether

Fig 2 demonstrates the same mechanism using inflation instead of

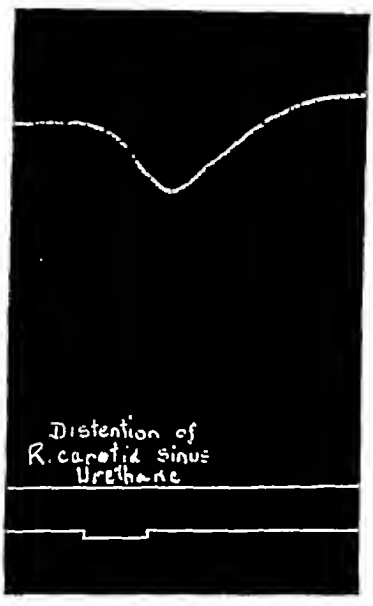


Fig 2 Fig 3

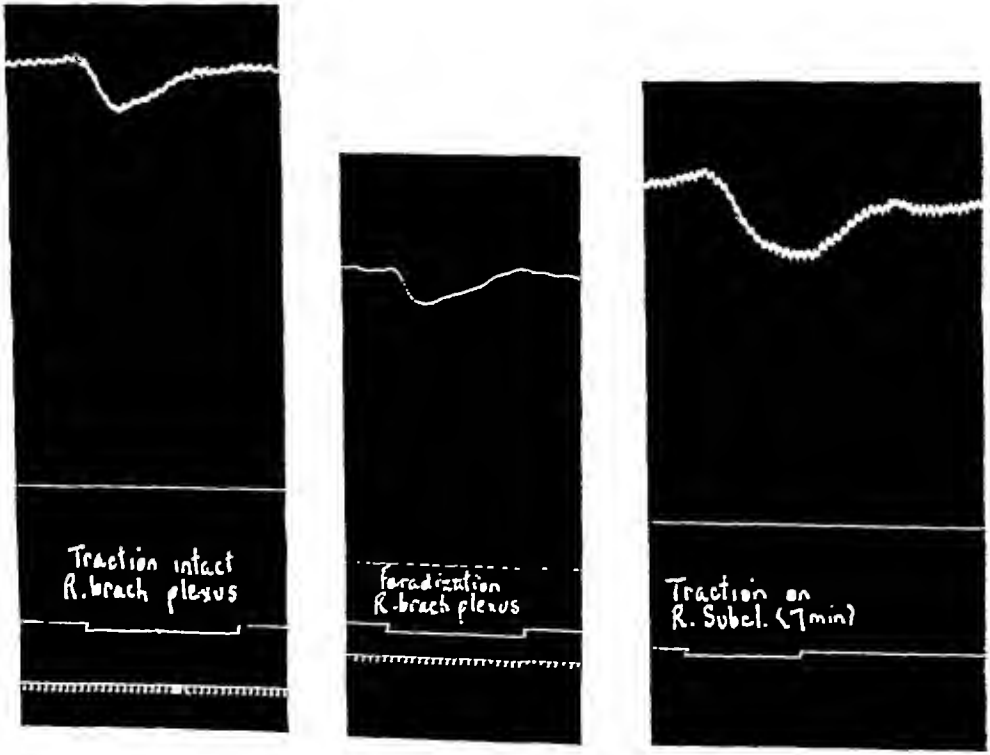
Fig 2 Rabbit Shows no effect from distension of the sinus caroticus under ether A pronounced fall from the same procedure when urethane is substituted

Fig 3 Rabbit. Urethane Traction on the central end of the cut brachial plexus Shows depressor effect and slowing of the heart Time in seconds

traction as a mode of stimulus In this experiment the internal carotid was tied as near the skull as possible The external carotid was tied well above the bifurcation and all the small arterial branches visible in the neighbourhood were ligated The sinus region was then inflated with saline through a syringe connected to a cannula inserted headwards into the common carotid artery Under ether anæsthesia there was no perceptible reaction during inflation When the change had been made to urethane a pronounced fall accompanied the distension It was also

found that traction on the central end of the cut brachial plexus would produce, under urethane, a well-marked fall of pressure This fall could be elicited after the removal of the stellate ganglion and the cutting of both vagi in the neck, or the administration of atropine However, with the vagi intact there was also a slowing of the heart (Fig 3) Under ether, traction on the plexus produced either a slight rise of pressure or no effect

An interesting point associated with this depressor effect is the spontaneous recovery of the blood-pressure level which takes place despite the continuance of the stimulus (stretch or faradisation, Fig 4)



a *b*

Fig. 4. Fig 5

Fig 4 *a* Rabbit. Urethane. Shows return to normal blood pressure during continuance of traction stimulation. *b* Rabbit Urethane. The same effect with faradic stimulation. Time in seconds

Fig 5 Rabbit. Urethane. Depressor effect from traction on the central end of the subclavian artery Atropine given 7 minutes beforehand.

Traction on the central end of the subclavian artery was also found to produce a fall of pressure under urethane (Fig 5) but this was analysed as being due to the traction on the artery being transmitted to the adjacent brachial plexus Traction on a muscle in the rabbit (opening the jaws or stretching the quadriceps femoris) gives no effect or a rise in blood-pressure under ether anæsthesia When however urethane has been substituted a depressor effect is obtained (Fig 6)

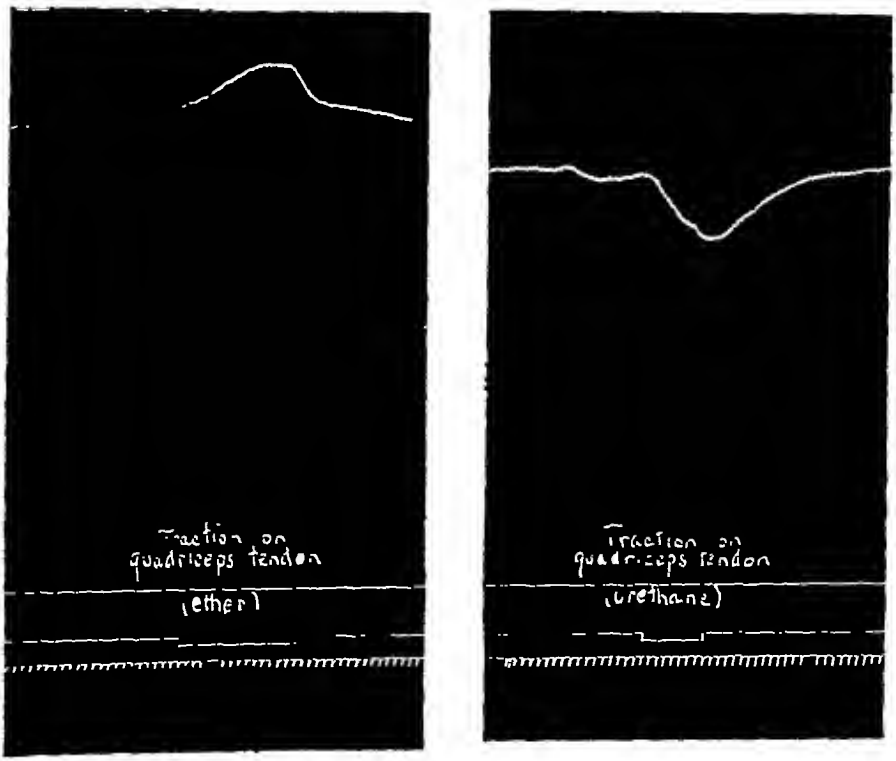


Fig 6 Rabbit. Under ether traction on the quadriceps femoris produced, in this case a rise of pressure When urethane was substituted for ether there was a fall of pressure from the same procedure Time in seconds

Fig 7 shows the result of faradisation of the central end of the femoral nerve with urethane anæsthesia There is a considerable fall of blood-pressure

It is clear, from the tracings and explanation given, that urethane, even when only producing light anæsthesia, is capable of influencing the rabbit's reactions so that depressor effects are obtained from a

variety of stimuli. The experiments herein described offer no explanation as to the mode of action of the urethane but, in view of the widespread

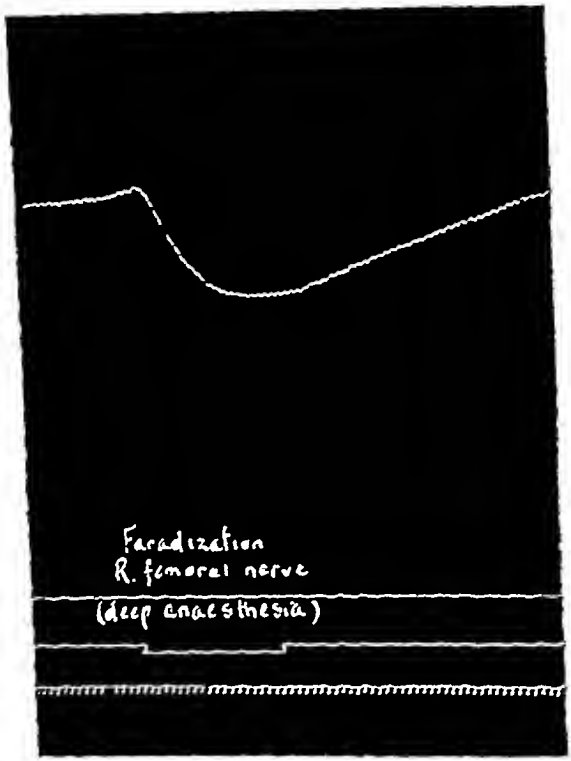


Fig 7 Rabbit. Urethane Shows the depressor effect from stimulation of the central end of the femoral nerve

use of this drug, it was thought desirable to call attention to this disturbing factor in the evaluation of results

SUMMARY

Under urethane anæsthesia a variety of stimuli, in the rabbit, produce depressor reflexes which are slight or absent under ether anæsthesia

This work was done by H. F while working under the Freedom Research Fund of the London Hospital.

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THE ORIGIN OF THE GLUCOSE IN THE HYPERGLYCÆMIA INDUCED BY PITUITRIN

By G A. CLARK

(*From the Physiological Laboratory, Sheffield University*)

The injection of extracts of the posterior lobe of the pituitary either intravenously or subcutaneously into a normal animal produces usually an increase in blood-sugar which may last from one to two hours or, in some cases, longer, depending on dosage, and the type and condition of animal, occasionally the hyperglycæmia is negligible and in a few instances the blood-sugar has been found to fall (1, 2) While it is accepted that the usual action of pituitrin[1] is to produce hyperglycæmia, there is no satisfactory evidence to show how the effect is brought about Moehlig and Ainslie (3), however, have suggested that the source of the additional sugar is the muscle glycogen, which they believe may be liberated in an indirect manner by the action of pituitrin on the suprarenals If pituitrin can set free glycogen from the muscles in this way, it appeared that the decerebrated, eviscerated animal was a suitable preparation in which to investigate the problem

The preparations were made from cats according to the method described by Burn and Dale (4) and the continuous glucose infusion apparatus of these authors was used to maintain as nearly as possible a constant blood-sugar level prior to the injection of pituitrin, glucose infusion was continued throughout the experiment at the same rate The concentration of the solution was 1 5 p c and the rate of infusion required varied in different animals from 1 c c in 2 9 mins to 1 c c in 4 mins The temperature of the preparation was maintained by means of an electrically heated table, hot water bottles and suitable felt wrappings which formed a tent over the animal and contained a warming bulb Blood for sugar estimations was withdrawn from one femoral vein and pituitrin injections were made into the femoral vein of the opposite limb The dose of pituitrin was in every case 1 c c

[1] The pituitrin used and referred to throughout the paper is the preparation issued under that name by Parke, Davis and Co

From Table I it is seen that in no experiment was the sugar level raised by pituitrin but, on the contrary, a very definite fall was produced This fall was not evident till the second half-hour in some cases while in others it was apparent during the first half-hour after pituitrin Control experiments in which no pituitrin was given showed no such abrupt changes in sugar level over the same period of time (Exps 7 and 8)

TABLE I.

		Before pituitrin			After pituitrin		
Exp	Hours	1	½	0	½	1	1½
1		330	320	310	266	231	—
2		242	238	230	219	206	194
3		294	291	288	288	259	—
4		250	253	254	250	206	197
5		204	210	218	223	210	200
6		418	419	426	412	346	325
7 (control)		326	321	314	311	305	296
8 (control)		262	265	265	267	264	270

In every case except Exp 5 the suprarenals and their blood-supply were left intact, in Exp 5 the glands were excluded from the circulation by a pedicle ligature, without in any way altering the subsequent response to pituitrin The sudden increase in the rate of fall of glucose concentration in this series of experiments may be explained by (*a*) dilution of the blood, (*b*) increased oxidation of glucose, or (*c*) building up or decreased breaking down of muscle glycogen

In support of the first of these possible explanations there are the observations of Craig(5) on human beings and of Underhill and Pack(6) on dogs that the administration of pituitary extract in conjunction with the exhibition of large quantities of water does cause a diminution in hæmoglobin content of the blood, while Partos and Katz-Klein(7) obtained a definite dilution of the blood in rabbits as a result of the injection of pituitary extract and showed that this dilution may mask the hyperglycæmia usually seen in the intact animal In the present series of experiments the hæmoglobin content of the blood was determined at intervals before and after pituitrin in three preparations and in two of them blood-sugar estimations were made at the same time The results are shown in Table II

It is obvious that in Exp 4 blood dilution cannot account for the observed fall in blood-sugar and that in Exp 9 the 5 p c dilution following the injection of pituitrin would be insufficient to account for the fall of sugar concentration in any of the experiments in Table I In Exp 6 where the concentrations of both sugar and hæmoglobin are seen to fall after the dose of pituitrin it is necessary to examine the changes

TABLE II.

Exp	Hours	Before pituitrin 1	½	0	After pituitrin ½	1	1½
4	Hæmogl. p c	68	70	69	68	67	64
	Glucose	250	253	254	250	206	197
6	Hæmogl p c.	88	87	85	82	75	73
	Glucose	418	419	426	412	346	325
9	Hæmogl. p c	—	60	57	56	54	54
	Glucose	—	—	—	—	—	—

more closely If curves are drawn to show the percentage change in the initial concentrations throughout the experiment (Fig 1) it is seen that before pituitrin was injected the sugar concentration was rising slightly and the hæmoglobin falling, both changes clearly the result of the infusion of sugar solution After pituitrin had been injected the sugar concentration fell more rapidly than that of the hæmoglobin[1] It is difficult to see how dilution of the blood by tissue fluid could account for this The fluid in the tissue spaces would contain no hæmoglobin, but at any rate some sugar, an amount of sugar, in fact, determined by the amount in the blood Unless, therefore, the capillaries can take up

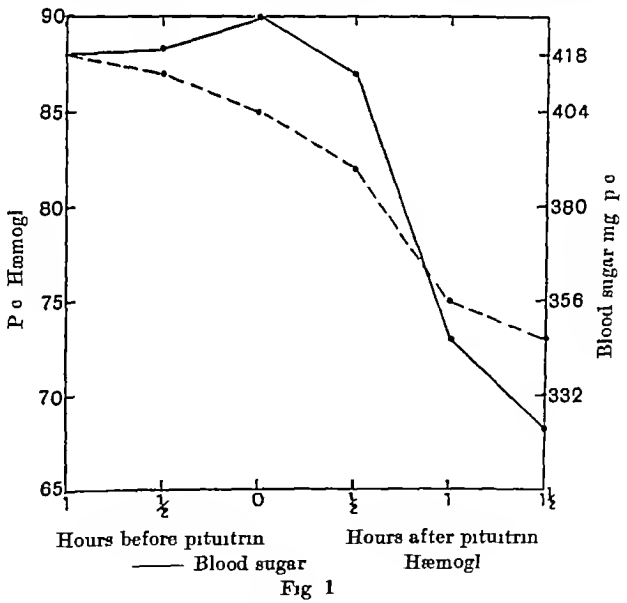

Fig 1

fluid from the tissue spaces in such a way as to exclude the sugar which that fluid contains, a supposition for which no evidence exists and which

[1] I am indebted to Mr A. Spencer for carrying out hæmoglobin estimations

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it is very difficult to allow, the dilution of the blood by tissue fluid must lower the concentration of hæmoglobin more than that of the sugar The figures in this experiment and the curves in Fig 1 show that, on the contrary, the concentration of sugar after pituitrin even in this experiment is lowered more than that of the hæmoglobin The fall of blood-sugar therefore cannot be accounted for by blood dilution Whatever be the cause of the disappearance of glucose from the blood in eviscerated animals following the injection of pituitrin, it is obvious that the muscles are not the source of the additional sugar in pituitrin hyperglycæmia Whether pituitrin has any specific action on carbohydrate metabolism in muscle is not known, but the experiments of Ahlgren(8) suggest the possibility Using Thunberg's methylene blue method for measuring tissue oxidation, he showed that some samples of hypophyseal extract definitely increased the oxidation processes in muscle, with other samples he found a decrease

The results so far described are of interest in view of the findings of Tingle and Imrie(9) that pituitrin temporarily lowers the blood-sugar in diabetics Moreover, in two of the cases reported by these authors there is a further similarity in that the fall was not evident till the second half-hour

Further confirmation of the view that pituitrin cannot liberate glucose from the muscles was obtained in three experiments on the decerebrated and eviscerated cat in an attempt to demonstrate the antagonism between pituitrin and insulin 1 c c of pituitrin was injected intravenously half an hour after the administration of 7 clinical units of insulin It is evident from Table III that in no case is the rate of fall of blood-sugar retarded by the pituitrin

TABLE III.

		Before insulin			After insulin		
Exp	Hours	1	½	0	½	1	1½
10		213	204	196	174	133	109
11		275	284	292	254	222	204
12		305	321	341	274	248	—

The abrupt rise in sugar level following the injection of pituitrin in the normal animal suggests a sudden glycogenolysis rather than a temporary suppression of the normal rate of utilisation of glucose It was natural therefore to consider the liver as the possible seat of action For this investigation cats under amytal anæsthesia were used, the liver was excluded from the circulation by anastomosing the portal vein with the

I wish to thank Professor Leathes for his unfailing interest and helpful criticism in this work

The expenses of this investigation have been defrayed by a grant from the Medical Research Council

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BILE SALTS AND SECRETIN AS CHOLAGOGUES

By J MELLANBY

(*From the Sherrington School of Physiology, St Thomas's Hospital, London*)

The hypothesis of the circulation of the bile is founded mainly on observations made by Schiff(1) in 1868 Schiff established, in dogs, a large fistulous opening between the surface of the abdomen and the gall bladder without the common bile duct being ligatured Under these circumstances the bile usually flows externally as long as the exit is free If, however, the cannula fitted into the fistula be closed, then the bile flows into the intestine Such fistulæ were called "amphibolic' by Schiff He found that the quantity of bile obtained in a given period from the fistula was always much greater immediately after the bile had been allowed to flow into the intestine than when it had been allowed to flow externally Further, he observed that the introduction of bile salts into the intestine through a duodenal fistula increased the total quantity of bile secreted Upon these facts Schiff based his hypothesis of the circulation of the bile—that a portion of the bile absorbed from the intestine, on reaching the liver in the portal blood, supplies the material for a fresh secretion of bile

In 1902 Bayliss and Starling(2) showed that the intravenous injection of secretin freed from bile salts caused not only a copious secretion of pancreatic juice, but also doubled the rate of flow of bile from the liver In the experiment quoted the spontaneous secretion of bile was 27 drops in 15 min , this was increased to 42 drops in 12 min after the injection of the secretin solution

In 1924 I(3) showed that the introduction of bile of an adequate reaction into the duodenum of a cat caused a large secretion of pancreatic juice, extending in some cases over a period of three hours An analysis of this phenomenon showed that the immediate stimulus to the pancreas was secretin which had been carried by the absorbed bile salt into the portal blood These observations indicated that the increased flow of bile observed by Schiff after the introduction of bile into the duodenum might have been due to the action of secretin on the liver

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into the duodenum of a cat The cat was in a fasting condition and, as previously described, it was necessary to add bicarbonate to the bile to evoke a secretion of pancreatic juice There was no spontaneous secretion of pancreatic juice, that of the bile was 10 drops per hr Thirteen minutes after the injection of the dilute bile, both pancreatic juice and bile were secreted at a rapid rate The following figures give a synopsis of the results

Time (hr)	Pancreatic juice (drops)	Bile (drops)
1st	70	58
2nd	54	62
3rd	9	15
Total juice—5·6 c.c in 3 hr		Total bile—5·0 c c. in 3 hr

After 3 hr the flow of pancreatic juice ceased and the bile flow diminished to its spontaneous rate—10 drops per hr The experiment offers an adequate demonstration of Schiff s statement that the entrance of the bile into the duodenum causes a flow of bile But it does not justify the conclusion that the injected bile is the immediate cause of the increased bile flow from the liver In fact the parallelism of the secretions of pancreatic juice and bile appear to suggest that both secretions are due to the same cause—the passage of secretin into the blood

(2) *The injection of bile into the ileum* The hypothesis that secretin is the immediate excitant of biliary secretion after the injection of bile into the duodenum was tested by direct experiment In the carnivorous animal the ileum is practically free from secretin. Therefore, if the absorption of bile injected into this part of the intestine is followed by an increased secretion of bile from the liver, it is evident that secretin cannot be regarded as the sole agent in the secretion of bile Slightly acidified bile (2 c c ox bile, 8 c c NaCl 0 9 p c , 0 05 c c HCl(N)) was injected into the ileum of a fed cat After 26 min. bile was rapidly secreted at the following rate

Bile (drops)	Min.	sec.
6	6	50
12	15	10
24	32	35
36	51	2
48	70	0
60	96	0

The increased secretion of bile continued for 2 5 hr during which time 3 c c of bile were secreted There was no secretion of pancreatic juice during the whole of this time. It is evident therefore that the liver

cells in a manner comparable to the action of secretin on the cells of the pancreas This hypothesis would correlate the observations of Schiff with those of Bayliss and Starling and would explain why substances so dissimilar as bile salts and secretin stimulate the liver cells to secrete bile

Experimental Methods The majority of the results recorded were obtained from experiments on cats In certain instances rats, which possess no gall bladder, were used as the experimental animals All the animals were anæsthetised by a hypodermic injection of veronal (0 4 grm per kilo of body weight) During the preliminary operations, chloroform was given, especially in the case of old cats in which the anæsthetic effect of veronal is delayed Cannulæ were tied into the gall bladder, the common bile duct, and the pancreatic duct Intravenous injections were made by means of a cannula tied into the right femoral vein and arterial blood-pressure records were obtained from the femoral or carotid artery The rates of secretion of bile and pancreatic juice were timed by a stop watch

The natural flow of freshly secreted bile in the cat is from the liver down the common bile duct If, however, the path down the common bile duct is obstructed, either through the contraction of the duodenal muscle round that portion of the duct which passes through the intestinal wall or through some other cause, then the bile is diverted along the cystic duct to the gall bladder When a cannula is placed in the common bile duct there is no obstruction to the flow and the freshly secreted dilute bile may be collected from it This fact is of importance since a fundamental difficulty in determining the factors responsible for the secretion of bile is the presence of a gall bladder, capable of considerable variations in capacity, on the path of the secretion The constancy of the level of bile in the cannula tied into the gall bladder showed that all the bile secreted by the liver passed directly down the common bile duct Results obtained from animals in which the secreted bile came from the gall bladder owing to some obstruction in the common bile duct were rejected The spontaneous variations in the capacity of the gall bladder are so considerable that such observations on biliary secretion over definite intervals of time are unreliable

Bile as a Cholagogue

(1) *The injection of bile into the duodenum* The following figures give the amounts of bile and pancreatic juice secreted after the injection of dilute bile (2 c c ox bile, 7 c c NaCl 0 9 p c , 1 c c $NaHCO_3$ 1 5 p c)

into the duodenum of a cat The cat was in a fasting condition and, as previously described, it was necessary to add bicarbonate to the bile to evoke a secretion of pancreatic juice There was no spontaneous secretion of pancreatic juice, that of the bile was 10 drops per hr Thirteen minutes after the injection of the dilute bile, both pancreatic juice and bile were secreted at a rapid rate The following figures give a synopsis of the results

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Total juice—5 6 c.c in 3 hr		Total bile—5 0 c.c in 3 hr

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secretes bile after the injection of bile into the small intestine quite independently of the absorption of secretin into the blood The result does not exclude secretin as a stimulus to the biliary activity of the liver, but it proves that the secretion of bile may be augmented independently of the passage of secretin into the blood

(3) *The intravenous injection of extracts of ileum* The previous results indicate that two secretins may exist, one for the pancreas, and the other for the liver, and that whereas the former is limited to the duodenum, the latter may extend along the whole length of the small intestine On this hypothesis bile salts absorbed from the duodenum carry both secretins into the blood and cause the secretion of both pancreatic juice and bile, whilst bile salts absorbed from the ileum carry the hepatic secretin only into the blood and hence cause the secretion of bile only This hypothesis was tested by making extracts of the mucosa of the ileum with various solvents (water, alcohol, dilute acid, dilute alkali, 0 9 p c NaCl, etc) and observing the effects of the intravenous injection of these extracts on biliary secretion In no case was there any increase in the bile secreted Exhaustive experiments gave no evidence in support of the hepatic secretin hypothesis

(4) *The intravenous injection of bile* The following experiment showed that the action of bile in the intestine as a cholagogue is directly due to the absorption of bile into the portal blood 1 c c of ox bile diluted with 5 c c of 0 9 p c NaCl was injected into the femoral vein of a cat The spontaneous secretion of bile was at the rate of 16 drops per hr

Time (min.)	A. Bile secreted (drops)	B Spontaneous secretion (drops)
15	8	4
30	23	8
45	35	12
60	46	16
90	53	24

Column B is inserted so that the amount of bile secreted after the intravenous injection of bile (column A) may be compared with the quantity which would have been secreted spontaneously owing to the continuous activity of the liver The figures show that the intravenous injection of bile (1 c c) increased the secretion of bile for 1 hr , a threefold increase being recorded in the first 45 min

(5) *The intravenous injection of sodium cholate* An analysis of the above experiment shows that cholalic acid is the substance present in bile which stimulates the liver to secrete more bile The effect on biliary

secretion of the intravenous injection of 0 1 grm of cholalic acid, dissolved in dilute NaOH, is given in the following figures

Time (min.)	Bile secreted (drops)	Spontaneous secretion (drops)
15	10	2
30	24	4
45	41	6
60	48	8

The enhanced secretion of bile terminated after 1 hr as in the previous experiment In these last two experiments the spontaneous secretion of bile was small since the cats were in a fasting condition.

The results of the experiments described in this section show that the absorption of bile salts (cholalic acid) from the intestine into the blood, apart from the synchronous absorption of pancreatic secretin (or hypothetical hepatic secretin), increases the amount of bile secreted by the liver The experiments fully confirm Schiff's original hypothesis of the circulation of the bile

Secretin as a Cholagogue

Bayliss and Starling showed that the intravenous injection into a dog of secretin contained in a 0 2 p c HCl extract of the duodenal mucous membrane doubled the secretion of bile by the liver Bile salts were removed from the duodenal mucous membrane by alcohol before making the secretin extracts My experiments indicate that secretin is contained in a preformed condition in the duodenal mucous membrane and is at least as soluble in alcohol as bile salts Therefore, in view of the marked cholagogue action of 0 1 grm of cholalic acid (cf *supra*), it appeared advisable to determine the capacity of purified secretin to cause a secretion of bile

(1) *Properties of purified secretin* I(4) have previously given a brief description of the method by which purified secretin may be obtained The preparation possesses a high degree of activity when tested on pancreatic secretion It contains no trace of bile salts and has no action on the general blood-pressure This latter fact is of importance since changes of blood-pressure that alter the amount of blood in the liver might directly bring about the retention or expression of bile from the bile canaliculi and ducts

(2) *The intravenous injection of secretin* This preparation of secretin, when injected into the blood stream in sufficient quantity to produce a large flow of pancreatic juice, augments to a small degree the secretion

secretes bile after the injection of bile into the small intestine quite independently of the absorption of secretin into the blood The result does not exclude secretin as a stimulus to the biliary activity of the liver, but it proves that the secretion of bile may be augmented independently of the passage of secretin into the blood

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(5) *The intravenous injection of sodium cholate* An analysis of the above experiment shows that cholalic acid is the substance present in bile which stimulates the liver to secrete more bile The effect on biliary

vein of a fasting cat The spontaneous secretion of bile was at the rate of 4 drops of bile per hr The quantities of pancreatic juice and bile, and the latent periods between the injection of secretin and the start of the secretion of pancreatic juice and the increased secretion of bile, are given

	Secretin (mgrm)	Pancreatic juice	Latent period (secs)	Bile	Latent period (min)
1st injection	0 5	46 drops in 30 min	50	13 drops in 32 min	10
2nd injection	0 5	52 drops in 26 min	45	13 drops in 45 min	10
3rd injection	0 5	54 drops in 28 min	45	14 drops in 42 min	5

The cessation of bile secretion after varying times (32 min , 45 min and 42 min), and the long latent period before a fresh injection of secretin initiated a renewed flow of bile, offered some support to the hypothesis of the mechanical expulsion of bile from the canaliculi in the liver

In order to obtain other evidence bearing upon this hypothesis an experiment was done in which injections of secretin (0 1 mgrm) were repeated every 30 min , that is, at intervals of time during which the apparent cholagogue action of secretin manifests itself On the mechanical hypothesis of the action of secretin successive injections, whilst producing the same quantity of pancreatic juice, should produce diminishing quantities of bile

Time of injection (p m)	Pancreatic juice (drops)	Bile (drops)
2 30	16	21
3 0	17	20
3 30	18	20
4 0	20	21
4 30	20	21

The spontaneous secretion of bile was at the rate of 10 drops in 31 min The constancy of the augmented secretion of bile (10 drops every 30 min) proved that this augmented secretion was not secondary to some mechanical action, either vascular or cellular, by which previously secreted bile contained in the bile canaliculi or ducts was squeezed out of the liver

(5) *Removal of small intestine* The long latent period which elapses between the injection of secretin and the augmentation of biliary secretion seemed to indicate that the cholagogue action was secondary to an effect on some other tissue An apparent increase in intestinal movements after the intravenous injection of secretin suggested that accelerated absorption into the blood of bile salts contained in the small intestine

of bile by the liver The small increase in the bile flow is invariably produced whether the spontaneous secretion of bile is large or small The following figures show the effect on the secretions of pancreatic juice and bile of an injection of 0 1 mgrm of secretin (dissolved in 1 c c H_2O) into the femoral vein

Time (min.)	Pancreatic juice (drops)	Bile (A) (drops)	Bile (B) spontaneous secretion (drops)
5	12	4	3
10	26	9	6
15	37	13	9
20	41	16	12
30	—	21	18

Column B shows the spontaneous secretion of bile on the assumption that the factors which cause this constant secretion persist after the intravenous injection of secretin The figures show that pancreatic juice was rapidly secreted for 20 min (41 drops), and during that time the secretion of bile was augmented by 4 drops The increased secretion of bile may be much less than that observed by Bayliss and Starling

(3) *The destruction of secretin* The question arose whether it was one and the same principle in the secretin preparation that excited the secretions of both pancreatic juice and bile, or whether two different secretins, pancreatic and hepatic, were contained in it It has been shown previously that pancreatic secretin is destroyed by pepsin, trypsin, or hydrolysis by weak acid or alkali The partial or complete destruction of the pancreatic action of the secretin preparation by any of the above means results in a corresponding change in the cholagogue action The results indicated that the same active principle causes the flow of pancreatic juice and the increase in the spontaneous secretion of bile

(4) *Repeated injections of secretin* Many observations suggested that the cholagogue action of secretin was apparent rather than real—that secretin caused a little bile to be squeezed out of the liver either by increasing the size of the liver cells or by altering to some small degree the amount of fluid (blood or lymph) contained in the liver The average weight of a cat's liver is 100 grm , whilst the increased secretion of bile, produced by a quantity of secretin which causes a maximal secretion of pancreatic juice, amounts to about 0 5 c c Therefore only small changes in the condition of the cells or fluid content of the liver would expel this extra quantity of bile from the canaliculi or ducts The type of experiment which supported this conclusion is given

0 5 mgrm of secretin was injected every 45 min into the femoral

vein of a fasting cat The spontaneous secretion of bile was at the rate of 4 drops of bile per hr The quantities of pancreatic juice and bile, and the latent periods between the injection of secretin and the start of the secretion of pancreatic juice and the increased secretion of bile, are given

	Secretin (mgrm.)	Pancreatic juice	Latent period (secs.)	Bile	Latent period (min.)
1st injection	0·5	46 drops in 30 min	50	13 drops in 32 min	10
2nd injection	0 5	52 drops in 36 min.	45	13 drops in 45 min.	10
3rd injection	0 5	54 drops in 38 min.	45	14 drops in 42 min.	5

The cessation of bile secretion after varying times (32 min , 45 min and 42 min.), and the long latent period before a fresh injection of secretin initiated a renewed flow of bile, offered some support to the hypothesis of the mechanical expulsion of bile from the canaliculi in the liver

In order to obtain other evidence bearing upon this hypothesis an experiment was done in which injections of secretin (0 1 mgrm) were repeated every 30 min , that is, at intervals of time during which the apparent cholagogue action of secretin manifests itself On the mechanical hypothesis of the action of secretin successive injections, whilst producing the same quantity of pancreatic juice, should produce diminishing quantities of bile

Time of injection (p m.)	Pancreatic juice (drops)	Bile (drops)
2 30	16	21
3 0	17	20
3 30	18	20
4.0	20	21
4 30	20	21

The spontaneous secretion of bile was at the rate of 10 drops in 31 min The constancy of the augmented secretion of bile (10 drops every 30 min) proved that this augmented secretion was not secondary to some mechanical action, either vascular or cellular, by which previously secreted bile contained in the bile canaliculi or ducts was squeezed out of the liver

(5) *Removal of small intestine* The long latent period which elapses between the injection of secretin and the augmentation of biliary secretion seemed to indicate that the cholagogue action was secondary to an effect on some other tissue An apparent increase in intestinal movements after the intravenous injection of secretin suggested that accelerated absorption into the blood of bile salts contained in the small intestine

was the immediate cause of the increased secretion of bile The assumption was tested by an experiment in which the cholagogue action of secretin, before and after removal of the small intestine, was determined The intravenous injection of 1 mgrm of secretin into a cat caused the secretion of 46 drops of pancreatic juice and 22 drops of bile in 40 min The small intestine was removed and the same quantity of secretin injected In the succeeding 40 min 34 drops of pancreatic juice and 14 drops of bile were secreted The amount of bile secreted after partial evisceration was diminished, but the diminution was of the same order as that of the pancreatic juice and was probably due to the shock caused by the removal of the small intestine which immediately preceded the second injection There was no evidence that the cholagogue action of secretin was the result of an increased absorption of bile salts from the intestine

(6) *Effect of clamping the pancreatic vein* As a corollary to the previous experiment, the effect of preventing or diminishing the blood flow through the pancreas, before and after the injection of secretin, was determined Two experiments are recorded in which (*a*) the pancreatic vein was ligatured and no collateral circulation was established in the pancreas, and (*b*) the pancreatic vein was clamped, but a collateral circulation was established after 15 min as evidenced by a small secretion of pancreatic juice

(*a*) In the following experiment the spontaneous secretion of bile, and the secretions of bile and pancreatic juice caused by the intravenous injection of 0 1 mgrm of secretin before and after the ligation of the main pancreatic vein are recorded

	Pancreatic juice (drops)	Bile (drops)
Normal flow for 30 min.	0	10
Secretion during 30 min after 0 1 mgrm of secretin	20	20
Pancreatic vein ligatured		
Secretion during 30 min. after 0 1 mgrm. of secretin	0	11

The occlusion of the main pancreatic vein does not prevent the access of secretin to the liver cells by the hepatic artery Since, under these conditions, secretin not only produces no pancreatic juice, but does not augment the spontaneous secretion of bile, it may be assumed that the prime action of secretin is on the cells of the pancreas, and that the cholagogue action is due to some product of pancreatic activity which passes via the blood to the liver and excites it to secrete bile

(*b*) This conclusion was confirmed by an experiment in which a collateral circulation was established after clamping the main vein

	Pancreatic juice (drops)	Bile (drops)
Normal flow for 1 hr	0	44
Flow in 1 hr after 0 2 mgrm. secretin	53	67
Pancreatic vein ligatured		
Flow in 1 hr after 0 2 mgrm. secretin	17	52

A collateral circulation through the pancreas was established 15 min after the injection of the second quantity of secretin since after this period, a small secretion of pancreatic juice started. The comparative figures bring out an interesting fact—the first secretin injection produced 53 drops of pancreatic juice and augmented the biliary secretion by 23 drops, the second secretin injection after clamping the pancreatic vein, produced 17 drops of pancreatic juice and augmented the bile secretion by 8 drops The quantities of pancreatic juice and bile bear about the same ratio to one another in both cases *i e* 53 to 17 and 23 to 8 The results indicate that the action of secretin as a cholagogue is a secondary effect depending upon its action on the pancreas Secretin acts on the pancreas causing a secretion of pancreatic juice, as a result of this glandular activity metabolic products pass into the portal blood and are carried directly to the liver, these metabolic products from the pancreas excite the biliary activity of the liver and cause an increased secretion of bile

Summary

(1) The injection of bile into the duodenum causes a large secretion of pancreatic juice and augments the secretion of bile

(2) A similar injection of bile into the ileum augments the secretion of bile but has no effect on pancreatic secretion.

(3) The intravenous injection of bile salts augments the flow of bile but has no effect on pancreatic secretion

(4) The intravenous injection of purified secretin causes a large secretion of pancreatic juice and augments the flow of bile

(5) The action of secretin as a cholagogue is annulled by procedures which prevent the secretion of pancreatic juice

(6) These facts show that (*a*) bile salts, free from secretin, absorbed into the blood from the small intestine act as cholagogues (*b*) the action of secretin as a cholagogue is secondary to its action on the pancreas

The expenses of this work were defrayed by a grant from the Government Grant Committee of the Royal Society

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THE SIGNIFICANCE OF THE DIASTOLIC AND SYSTOLIC BLOOD-PRESSURES FOR THE MAINTENANCE OF THE CORONARY CIRCULATION

By G V ANREP and B KING

(*From the Physiological Laboratory, Cambridge*)

It is generally agreed that the arterial blood-pressure is the main circulatory factor determining the coronary blood flow, but as regards the relative importance of the diastolic and systolic pressures opinions differ. Starling and his co-workers(1, 2, 3) find that the coronary circulation is within wide limits independent of changes in pulse pressure provided that the *mean* aortic pressure is kept constant On the other hand, Smith, Miller and Graber(4) state that the maintenance of an efficient coronary circulation is fundamentally dependent on the height of the *diastolic* pressure, and that only in experiments in which the diastolic pressure is reduced to a permanent low level can the coronary blood flow be affected by changes in the systolic pressure Starling's measurements of blood-pressure were in most cases made with the mercury manometer Smith, Miller and Graber used a membrane manometer the vibration frequency of which was not stated, but, as can be judged from the tracings given in their paper, it was probably not high

The determination of the relative significance of the systolic and diastolic blood-pressures is of considerable importance for a correct comprehension of the blood supply to the heart muscle It is especially important in conditions in which the pulse pressure changes without a material alteration in the mean pressure as may occur, for instance, as a result of acceleration of the heart beat or of a reduction in the arterial resistance with a simultaneous increase in the systemic output or in the case of aortic regurgitation

The experiments described below were performed with the object of securing further information on this point They were performed on the heart-lung preparation, since in this preparation the diastolic and systolic pressures can be easily and independently controlled and maintained constant for any desired length of time

The blood-pressure was measured immediately above the aortic valves with an optical manometer, the membrane of which had in the different experiments a vibration frequency of 150–170 and a sensitivity such as to give, at the magnification used, a deflection of 1 mm for each mm Hg change in pressure The blood flowing from the drained coronary sinus was collected and measured during intervals of 1 min and was considered constant if five successive measurements differed by not more than ± 0 5 c c The diastolic and systolic pressures were changed by varying (*a*) the systemic output or (*b*) the heart rate which was in all experiments controlled by rhythmic stimulation of the right auricle The actual experiments proceeded as follows Optical records of the aortic pressure were obtained after carefully measuring the coronary blood flow and the systemic output of the heart The output was then increased while the artificial arterial resistance was adjusted so as to maintain (say) the systolic pressure at the level it had during the preceding period of small output The aortic pressure and the coronary blood flow were again recorded The observation was then repeated, keeping in this case the diastolic pressure constant Now, still maintaining a large output, the arterial resistance was adjusted to a level at which the coronary blood flow was within ± 0 5 c c the same as it had been during the period of the small output, a further set of optical records of the aortic pressure being taken We thus had four sets of records of the aortic pressures and of the coronary blood flow in each experiment, the first (1) which was taken during the period of small output and the rest (2, 3 and 4) during the period of larger output, (2) with the same systolic pressure, (3) with the same diastolic pressure and (4) with an aortic pressure such that the coronary blood flow during the period of large output was equal to that during the period of small output In order to determine whether the condition of the coronary blood vessels had undergone a change during the 15 to 20 min of observation, the output and pressure were again returned to their original values, only those experiments being used in which the coronary blood flow during this control period was equal to that observed before the increase in the output The above four sets of observations could usually be repeated several times on the same heart-lung preparation Similar observations were made in experiments in which the pulse pressure was changed by varying the heart rate while the systemic output was maintained constant

The Effect of Changes in Output

The results of our experiments with changes in output were concordant in showing that neither the diastolic nor the systolic pressure alone determines the coronary circulation Thus our observations fail to support the conclusions reached by Smith, Miller and Graber, we find that the mean aortic pressure is in every case a far more significant factor in the coronary circulation Table I gives the results of a typical experiment

Table I.

Dog—10 kl., heart—62 grm The heart rate was maintained throughout this experiment at 122 beats per min. and the temperature at 37° C

Systemic output in c c. per min.	Diastolic pressure in mm. Hg	Systolic pressure in mm. Hg	Arithmetical mean pressure in mm Hg	Blood flow from the coronary sinus in c c. per min.
(a) The diastolic pressure is maintained constant				
1170	80	179	129½	142
890	80	168	119	99
465	80	130	105	68
200	80	118	99	57
(b) The systolic pressure is maintained constant.				
200	80	118	99	56
460	65	118	91½	42
885	42	118	80	31
1160	20	118	69	18
(c) The coronary blood flow is maintained constant				
200	80	118	99	57 0
470	70	126	99	56·5
880	60	134	97	56·5
1175	42	147	95	57·0

The first and the second part of the table show that the coronary outflow is affected by changes in the systolic pressure as well as by changes in the diastolic pressure or, in other words, that it is not determined by either of these pressures singly The coronary blood flow closely follows the changes in the arithmetical mean pressure, a rise in which has a relatively greater effect as the pressure reaches a higher level a change in the mean pressure from 99 to 129 mm increased the coronary flow by 85 c c while an equal change from 69 to 99 mm increased it by only 38 c c In the third part of the table the pulse pressure is progressively increased while the aortic resistance is so adjusted as to maintain the coronary outflow constant It can be seen that the mean aortic pressure had to be dropped by 4 mm. Hg in the case of the largest systemic output If, in this case, the mean aortic

pressure had been maintained constant, the coronary blood flow would have been a few cubic centimetres larger For instance, when after the last observation recorded in Table I (*c*) the mean pressure was increased from 95 to 99 mm Hg the coronary blood flow increased from 57 c c to 64 c c per min

Registration by the hot-wire method (5, 6, 7) shows that the coronary blood vessels are supplied with blood during the whole period of diastole, and that therefore all changes of pressure occurring after the beginning of relaxation of the ventricles must affect the coronary blood flow The coronary blood flow through the left ventricle is to all evidence completely arrested during systole It is highly probable, however, that the coronary supply to the right ventricle is not stopped by its less powerful contraction, and if this be the case then it should be expected that not only the post-dicrotic changes in the blood-pressure but also the changes occurring during systole must affect the coronary blood flow These complex relations are rendered still more difficult of analysis by the fact that the higher the maximum value of the blood-pressure the greater is its effect upon the coronary circulation, and also by the fact that the length of systole and the rate of relaxation of the heart change with alterations in

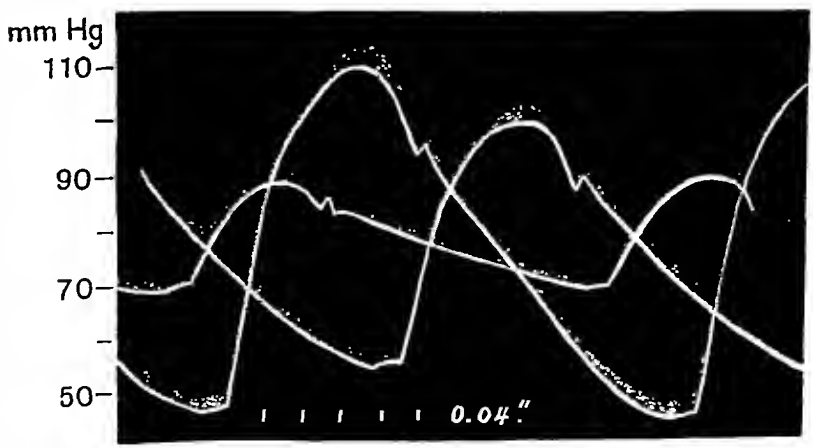

Fig 1 Record of the aortic blood pressure taken at different systemic outputs and constant coronary blood flows. The average arithmetical pressure is nearly the same in the three cases, while the diastolic pressure is widely different This fig shows the method of retracing used in the calculation of the pressures

the output On account of these complications it is not possible, at present, to evaluate more precisely the significance of changes in the

aortic pressure for the maintenance of the coronary blood flow Calculating by graphic methods from the areas of the curves the true mean pressures prevailing during the whole cycle or during the period of diastole, we found that these gave no more definite relations to the coronary blood flow than the arithmetical mean pressure The latter can be, therefore, taken as the one determining the coronary blood flow with such accuracy as our present methods allow Fig 1 gives a tracing of the aortic pressure as recorded in one of the experiments, Figs 2 and 3 are redrawn superimposed tracings of the aortic pressure

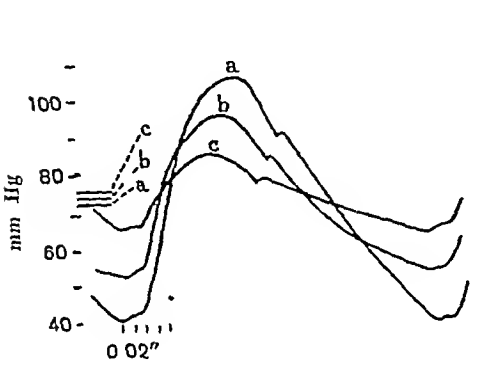

Fig 2

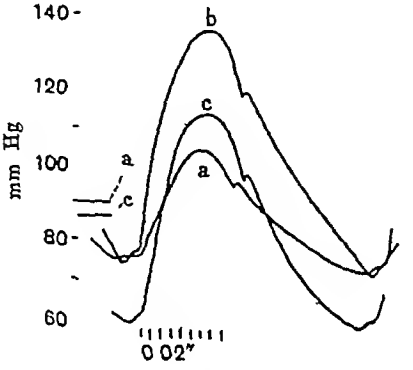

Fig 3

Fig 2 Redrawn superimposed tracings of the aortic blood pressure The systemic output in *a*, *b*, and *c* was 960, 520, and 250 per min. respectively The blood flow from the coronary sinus was constant at 37 5 c c. per min. The arithmetical average pressures are 73, 74 5 and 76 mm. Hg in *a* to *c* respectively

Fig 3 Redrawn tracings of the aortic blood pressure

a systemic output	300 c.c.,	coronary sinus blood flow		41 c.c
b ,,	930 c.c.	,,	,,	115 c c.
c ,	950 c c	,,	,,	41 c.c

The diastolic pressure is maintained constant in *a* and *b* The coronary blood flow is maintained constant in *a* and *c*.

The Effect of Changes in the Heart Rates

It has been reported on several occasions (1, 2, 3) that in the heart-lung preparation changes in the heart rate have no effect upon the coronary blood flow Drury (8) has made similar observations in his experiments upon the tortoise heart and Hammouda and Kinosita (9) upon the perfused rabbit's heart In a recent preliminary communication Miller, Smith and Graber (10) report that in experiments in which a rapid and regular action of the heart followed the stimulation of the auricle the rate of coronary flow was usually increased, while irregular action with

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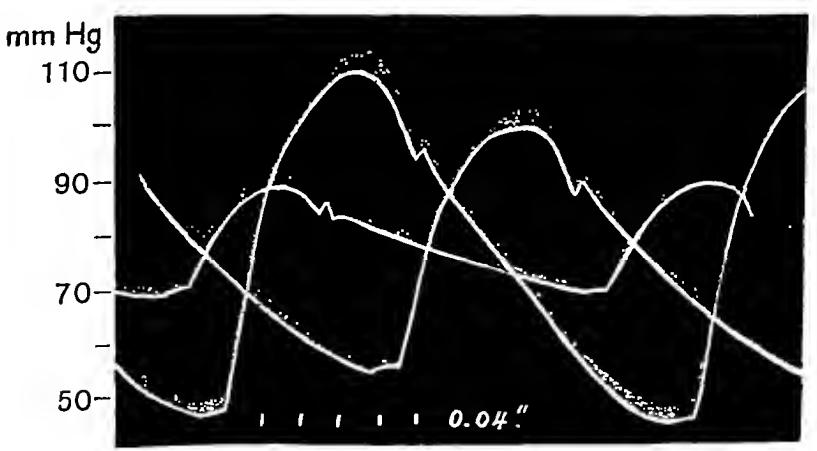

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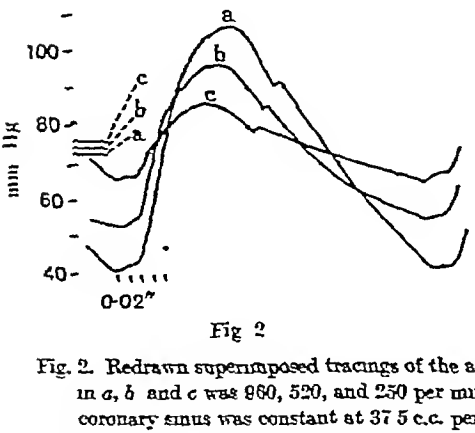

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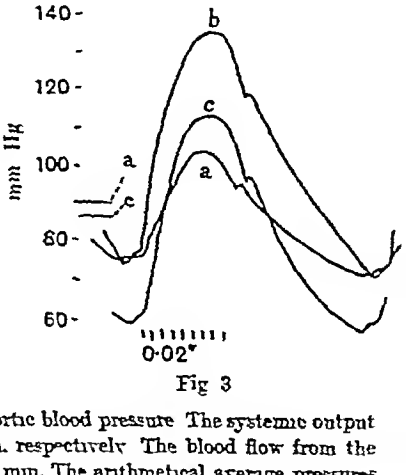

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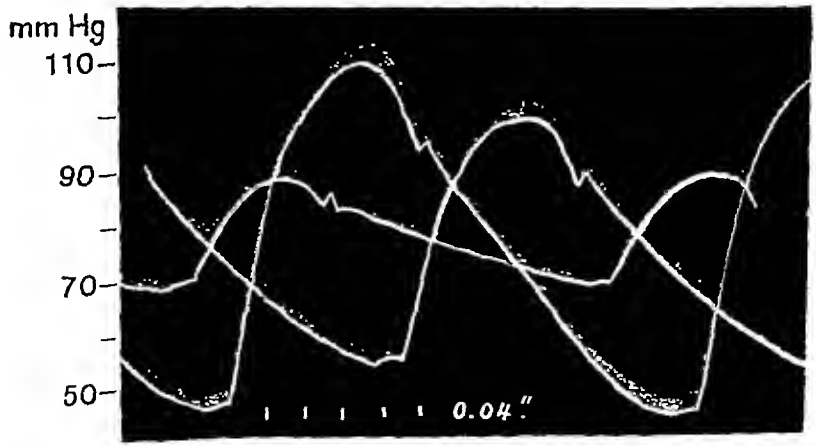

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2 The coronary blood flow closely follows the changes in the arithmetical average of the systolic and diastolic aortic pressures, which is in most cases a sufficiently accurate measure of the true mean pressure

3 Changes in the systemic output and changes of the heart rate have within wide limits no direct effect upon the coronary blood flow

The expenses of this research were defrayed by a grant from the Medical Research Council held by one of us (G V A.)

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the smaller the output This effect can be neglected in the experiments so far described in which the aortic pressure never became equal to the external pressure determining the arterial resistance, since neither the output nor the heart rate was excessively diminished But it does come into evidence in experiments in which the heart beat is reduced to a very slow rate, especially when the output per beat is small so that the pressure in the arterial system quickly falls towards its diastolic value For example, we have experiments with a small output in which the temperature of the blood was lowered to 33–34° C , the natural heart rate being reduced in consequence to about 40–50 beats per min The heart could be, however, accelerated by stimulation of the auricle to 90–115 beats per min In this case, in order to maintain the coronary blood flow constant the arithmetical mean aortic pressure had to be raised at the slower heart rates by a few millimetres On inspecting the aortic pressure curves it becomes evident that this apparent influence of heart rate is not due to a direct effect but to the fact that the arithmetical mean pressure ceases to give an approximate representation of the true mean pressure prevailing in the aorta On calculating the true mean pressure it was found to be nearly constant in conditions in which the coronary blood flow was constant (Table III)

TABLE III.

Dog—9 kl., heart—52 grm., Output—200 c c. per min., Temperature 33° C

The coronary blood flow is maintained constant.

Heart rate per min.	Diastolic pressure in mm Hg	Systolic pressure in mm. Hg	Arithmetical mean pressure in mm. Hg	True mean pressure in mm. Hg	Coronary flow from sinus in c c. per min.
55*	60	140	100	86	39 0
75	66	118	92	86	40 0
100	70	104	87	87	39 5

* Natural heart rate

The result of the experiments upon the effect of output and of heart rate on the coronary blood flow shows that changes in the strength of contraction of the heart as well as changes in the heart rate have no direct influence upon the coronary circulation The changes in the coronary blood flow which may be observed under these conditions are brought about indirectly through alteration of the aortic blood-pressure

CONCLUSIONS

1 The coronary blood flow is not determined by either the diastolic or the systolic aortic blood-pressure singly

2 The coronary blood flow closely follows the changes in the arithmetical average of the systolic and diastolic aortic pressures, which is in most cases a sufficiently accurate measure of the true mean pressure

3 Changes in the systemic output and changes of the heart rate have within wide limits no direct effect upon the coronary blood flow

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THE EFFECT OF ATROPINE, ERGOTAMINE, AND PITUITRIN ON PHLORHIZIN GLYCOSURIA

By A B ANDERSON and M D ANDERSON

(*From the Biochemical Laboratory, Cambridge*)

It has been suggested in several recent papers that phlorhizin produces glycosuria by acting on the sympathetic nerve endings in the kidney This suggestion is based on observations of the effect on phlorhizin glycosuria of drugs that paralyse the sympathetic nerve endings Thus Teschendorf(1) reports a decrease in sugar excretion after the administration of ergotamine to phlorhizinised rabbits Beck(2) finds a decreased excretion of sugar after the administration of ergotamine to children treated with phlorhizin Brogsitter and Dreyfus(3) report that the sugar excretion of phlorhizinised animals was inhibited by atropine

In all these cases the doses of phlorhizin given were small, and the animals were never fully phlorhizinised In view of the proved decrease in the secretion of urine produced by the drugs atropine and ergotamine, a decrease in the excretion of sugar caused by these drugs under the conditions described is not sufficient proof of an action antagonistic to that of phlorhizin Some better standard of comparison is required Such a standard is provided in the constant D/N ratio in the urine of completely phlorhizinised animals on a carbohydrate free diet For it is reasonable to suppose that if the action of the drug is directly antagonistic to that of phlorhizin, the D/N ratio will fall and less sugar will be excreted, whereas if the drug only decreases the secretion of urine, less sugar will be excreted, but at the same time the excretion of nitrogen will fall and the D/N ratio will be unchanged

In order to investigate the action of atropine and ergotamine on these lines, rats were phlorhizinised until a constant D/N ratio was obtained, and then the drug was administered The rat was chosen as the experimental animal partly for convenience in handling, and also because the technique for establishing a constant D/N ratio in the rat had already been worked out in connection with previous experiments not reported here

The animals were phlorhizinised by the method given by Coolen(4)

for dogs, 50 mg of phlorhizin suspended in olive oil was injected subcutaneously every day A satisfactory synthetic carbohydrate free diet was found to consist of protein 75 p c, fat 20 p c, salts 5 p c On this diet a constant D/N ratio was established after three or four days the level varying slightly with individual rats The urine was collected free from fæces in the usual manner over 24 hr periods Total nitrogen was determined by Kjeldahl's method after removing any protein contamination from spilt food by precipitating with trichloracetic acid and filtering The reducing power was estimated by the method of Wood-Ost after precipitating any contaminating protein with metaphosphoric acid Occasionally the urine was contaminated with a very little food

Atropine

Atropine in the form of the sulphate was dissolved in normal saline and administered by subcutaneous injection The results are given in Table I All the animals were phlorhizinised for three or four days before the first day given in the table, which is the first day on which a constant D N ratio was established

Table I.

Rat and weight before expt.	Day No	Urine vol c.c.	Glucose % gm.	Glucose total gm.	Nitrogen per c.c. mg	Nitrogen total mg	D/N	Remarks
EF 201 gm.	1	28	5·31	1·47	16·68	467	3·2	
	2	52	6·27	3·26	19·51	1015	3·2	
	3	41	6·33	2·59	18·95	777	3·3	
	4	31	6·23	1·93	18·27	566	3·4	5 mg atropine sulphate
	5	44	5·16	2·27	15·48	681	3·3	
	6	38	7·17	2·72	21·67	823	3·3	
EA 230 gm.	1	60	4·14	2·48	16·00	960	2·6	
	2	57	4·73	2·70	18·20	1037	2·6	
	3	22	7·23	1·59	22·05	485	3·3	
	4	11	8·40	0·92	23·71	261	3·5	25 mg atropine sulphate
	5	40	4·98	1·99	18·38	735	2·7	
EC 258 gm.	1	38	6·30	2·39	20·42	776	3·1	
	2	40	7·23	2·89	22·69	908	3·2	
	3	27	7·37	2·05	20·92	565	3·8	
	4	(6)	5·40	(0·32)	7·84	(47)	6·9	50 mg atropine sulphate. Rat died
EB 252 gm.	1	(41)	5·93	(2·43)	19·47	(798)	3·05	
	2	48	6·17	2·96	18·79	902	3·3	
	3	39	6·76	2·64	20·65	805	3·3	
	4	21	7·15	1·50	19·51	410	3·7	
	5	(11)	6·11	(0·67)	10·52	(116)	5·8	100 mg atropine sulphate. Rat died

Brackets indicate that the sample does not represent a collection over the full 24 hours

In the above table the D/N ratios are calculated from the figures given for the nitrogen per c c and the glucose per 100 c c The doses of atropine are recorded opposite the first sample of urine to be collected after injection of the drug, that is opposite the day on which the first effects of the drug might be observed The 24 hr samples of urine were collected

THE EFFECT OF ATROPINE, ERGOTAMINE, AND PITUITRIN ON PHLORHIZIN GLYCOSURIA

By A B ANDERSON and M D ANDERSON

(*From the Biochemical Laboratory, Cambridge*)

It has been suggested in several recent papers that phlorhizin produces glycosuria by acting on the sympathetic nerve endings in the kidney This suggestion is based on observations of the effect on phlorhizin glycosuria of drugs that paralyse the sympathetic nerve endings Thus Teschendorf(1) reports a decrease in sugar excretion after the administration of ergotamine to phlorhizinised rabbits Beck(2) finds a decreased excretion of sugar after the administration of ergotamine to children treated with phlorhizin Brogsitter and Dreyfus(3) report that the sugar excretion of phlorhizinised animals was inhibited by atropine

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In order to investigate the action of atropine and ergotamine on these lines, rats were phlorhizinised until a constant D/N ratio was obtained, and then the drug was administered The rat was chosen as the experimental animal partly for convenience in handling, and also because the technique for establishing a constant D/N ratio in the rat had already been worked out in connection with previous experiments not reported here

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255 gm.	2	49	7·23	2·89	22·69	908	3·2	
	3	27	7·37	2·05	20·92	565	3·8	50 mg atropine sulphate.
	4	(0)	5·40	(0·32)	7·84	(47)	6·9	Rat died
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from 9 30 a m and the atropine injected at 6 p m , *e g* in the case of rat *EF* the figures opposite the fourth day concern the sample of urine collected between 9 30 a m on the third day and 9 30 a m on the fourth day, and the atropine was injected at 6 p m on the third day

The figures for the D/N ratio show in all cases a rise after atropine, though in the case of rat *EF* the rise after 5 mg of atropine was negligible There was a fall in the quantities of urine, total nitrogen, and total glucose In the case of rats *EC* and *EB* doses of 50 and 100 mg of atropine respectively were fatal, the animal dying within 48 hours of the injection According to Clark(5) the toxic dose of atropine for rats varies from 500 mg per kilogram for young animals to 1 0 gm. per kilogram for adult animals If Clark's doses are of general application phlorhizinisation appears to lower the resistance of the animal to atropine

Ergotamine

Ergotamine in the form of tyramine acid phosphate was dissolved in normal saline and administered by subcutaneous injection The results are given in Table II The remarks on the methods of experiment and estimation which were made under the heading of atropine apply to these experiments

Table II.

Rat and weight before expt.	Day No	Urine vol c.c.	Glucose % gm.	Glucose total gm.	Nitrogen per c.c mg	Nitrogen total mg	D/N	Dose of tyramine acid phosphate
EG 176 gm.	1	38	5 43	2·06	17 47	664	3 11	
	2	41	5 76	2 36	19 18	786	3 00	
	3	43	5 37	2 31	18 83	810	2 85	1 mg
	4	39	5 70	2 22	19 29	752	2 95	
	5	38	5 70	2 17	19 29	733	2 95	
	6	35	6·00	2 10	18 72	655	3 20	5 mg
	7	42	6·24	2 62	19·40	815	3 22	
	8	27	7 77	2 10	23 83	643	3 26	
	9	43	6 49	2 79	20·88	898	3 11	10 mg
	10	52	5 88	3 06	19 79	1029	2 97	
EH 157 gm.	1	20	5 25	1 05	16 91	338	3 10	
	2	33	5 10	1 68	17 52	578	2 91	
	3	31	6 00	1 86	21 79	675	2 75	
	4	26	6·45	1 68	22·01	572	2 93	
	5i	18	4 77	0·86	14 80	266	3 22	40 mg in four doses at three hour intervals
	5ii	12	5 43	0·65	20 08	241	2 70	
	6	31	5 99	1 86	22 35	648	2 71	
	7	34	6·83	2 32	23 19	788	2 95	
EJ 170 gm.	1	35	6 21	2 17	22 39	784	2 77	
	2	29	6 84	1 98	22 35	648	3·06	
	3	38	6 99	2 66	21 44	815	3 26	
	4	32	7 43	2 38	22 47	719	3 31	100 mg
	5	41	6 87	2 82	22 35	916	3·08	

Doses of 1, 5, and 10 mg of tyramine acid phosphate appeared to have very little action in rat *EG* and the D/N ratio was hardly affected, the

very slight drop in D/N with a dose of 1 mg is counterbalanced by a slight rise with a dose of 5 mg In the case of rat *EH* on the fifth day the urine was collected in two twelve hour periods, in the first period 40 mg of ergotamine were given in 10 mg doses at three hour intervals, this dose caused a slight rise in D/N for the first period with a corresponding fall in the second so that the average for the two periods shows no change A dose of 100 mg given to rat *EJ* produced a similar slight rise followed by a slight fall for the next day

Pituitrin

No account of experiments on the action of pituitrin on phlorhizin glycosuria occurs in the literature This is a matter for some surprise in view of the striking effects of pituitrin on the secretion of the urine, *e g* the well-known effect of pituitrin in diminishing the diuresis after excessive water-drinking or in diabetes insipidus We have investigated the effect of pituitrin on phlorhizin glycosuria by two different methods in the first the drug was administered to the completely phlorhizinised animal with a constant D/N ratio as in the experiments with atropine and ergotamine, and in the second to the animal on a complete diet (that is a stock diet of protein, fat, carbohydrate) with daily injections of phlorhizin in 0 25 p c sodium carbonate solution The sterile standardised solutions of pituitrin prepared by Parke Davis and Co were used in these experiments Some of the results obtained are given in Table III

Table III.

Rat and weight before exp.	Day No	Urine vol. c.c.	Glucose % gm.	Glucose total gm.	Nitrogen per c.c. mg	Nitrogen total mg.	D/N	Remarks
			On protein-fat diet, phlorhizin 50 mg in oil daily					
EH	1	31	5·99	1·86	22·35	6·48	2·71	
157 gm.	2	34	6·83	2·32	22·19	7·55	2·95	
	3	31	6·48	2·01	22·01	6·82	2·94	
	4	35	6·51	2·28	22·01	7·70	2·86	Pituitrin 0·3 c.c.
	On stock diet		protein-carbohydrate-fat, phlorhizin 60 mg in Na_2CO_3 daily					
PJ	1	15	4·6	0·69	18·2	2·73	—	
183 gm.	2	17·5	3·4	0·595	14·1	2·47	—	
	3	21	5·6	1·176	12·5	2·62	—	
	4	14	6·2	0·868	18·4	2·57	—	Pituitrin 0·2 c.c.
	5	9	6·7	0·603	18·1	1·62	—	Pituitrin 0·3 c.c.

Rat *EH* on a protein-fat diet with a constant D/N ratio showed no alteration when injected with 0 3 c c pituitrin. In the case of rat *PJ* which was receiving the stock diet and 60 mg of phlorhizin in Na_2CO_3 daily, the injection of 0·2 c c and 0 3 c c pituitrin on the fourth and fifth days respectively caused a fall in the volume of urine and in the

total nitrogen, while the percentage of sugar in the urine rose The total sugar was not affected to any great extent As there were large variations in the total daily excretion of sugar before the injection of pituitrin, no very strict comparison of the total sugars before and after pituitrin could be made

Discussion

Judging from the D/N ratio in the above experiments atropine has no action antagonistic to that of phlorhizin This is contrary to the findings of Brogsitter and Dreyfus mentioned earlier These authors injected atropine and phlorhizin into rabbits and observed the sugar excretion There was a complete stoppage of urine for two to three hours and the first sample collected after the stoppage always contained sugar, often in greater amounts than the corresponding sample from the control animal receiving phlorhizin alone They then gave urea as a diuretic to overcome the anuria caused by atropine, and obtained a larger excretion of sugar which was, however, less than the amount excreted by the control animal The restricted secretion of urine in all these experiments might be sufficient to explain the lessened excretion of sugar after phlorhizin, because with the diminished secretion some of the phlorhizin which would normally pass through the kidney, producing its effect on the cells, might be excreted or destroyed in some other part of the body, again, the increased secretion of urine produced by urea after atropine is not comparable with the secretion of the same amount of urine by the normal kidney

In 1911 Gargiulo(6) reported that atropine arrested phlorhizin glycosuria in the rabbit but not in the frog, this also suggests that the action of atropine in checking sugar excretion is merely a consequence of the arrest of the kidney function

That atropine may have some effect on carbohydrate metabolism appears from the finding of Rafael(7) that glycosuria may be a consequence of atropine administration in normal animals Some such effect may be involved in the rise in D/N ratio observed after atropine in our experiments

Turning to ergotamine, similar objections may be raised to the experiments of Teschendorf (*loc cit*), who admits that there was a large decrease in water excretion in his experiments He argues that since the decrease in sugar excretion is much greater than the decrease in water elimination, it does not appear probable that the diminution in sugar excretion was due to the decreased water excretion In all our

experiments the D/N ratio was increased if anything after the administration of ergotamine and there is no evidence of a decrease in the sugar excretion The experiments of Adlersberg and Roth(8) may be quoted in this connection they found that the glycocholia produced by phlorhizin was not affected by ergotamine

The combined evidence of our experiments with atropine and ergotamine contradicts the theory that phlorhizin causes glycosuria by acting on the sympathetic nerve endings of the kidney

Summary

1 Atropine and ergotamine are found to have no definite effect on the D/N ratio in fully phlorhizinised rats on a protein-fat diet

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A DOUBLE PERFUSION-PUMP

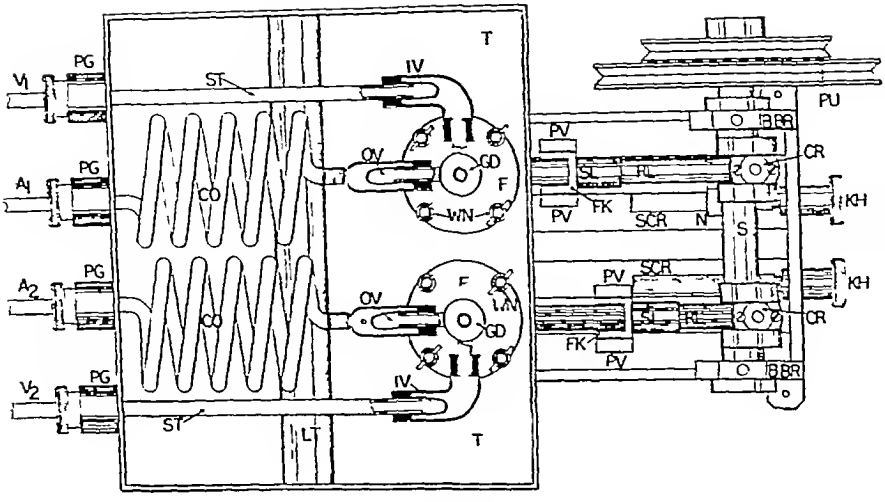
By H H DALE and E H J SCHUSTER

(*From the National Institute for Medical Research, Hampstead*)

The heart-lung preparation of Starling and his co-workers has provided a means, extensively used, of studying the functions of the mammalian heart when working against an artificial and adjustable peripheral resistance, which replaces the natural resistance of the systemic vascular system Many schemes have been devised and used, on the other hand, in which the blood has been perfused through the living vessels of an organ or part of the body by use of an artificial pump Our original object in devising the pump here described was to produce an adjustable mechanism which could be used to replace the heart, and to carry on both major and minor circulations of the whole body This would obviously require two synchronously working pumps Since the two pumps would be working against widely different resistances it was further important that their throws should be rapidly and independently adjustable while running, so as to maintain such equality of output on the two sides as would prevent accumulation of blood on one side of the system, with deleterious back-pressure on the capillaries of the lungs or of the rest of the body, as the case might be If the pumps and tubular connections were perfectly non-distensible, it should, theoretically, be possible to dispense with such adjustment, the two pumps being set to deliver equal amounts per stroke, they should continue to do this whatever changes occurred in the relation of the pressures they had to overcome In practice, however, it would be very difficult to make a system of such ideal rigidity The apparatus which we designed, in which diaphragm-pumps were used, certainly would not have it The arrangement was accordingly devised which enabled the throw of either pump lever to be increased or decreased quickly and adjusted with delicacy Embley and Martin (This *Journal*, 32 p 147 1905) used two rubber bulb-syringes, compressed by adjustable rotating cams, for a similar purpose Our apparatus should give greater rapidity and precision of control, but the principle is the same

Description of the Double Perfusion-Pump

The pump is shown in plan in Fig 1 and in elevation in Fig 2 Both drawings are sectional where this method of presentation has been considered necessary to demonstrate the relations of the working parts The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LN
BBR
V2
LT

Fig 1.

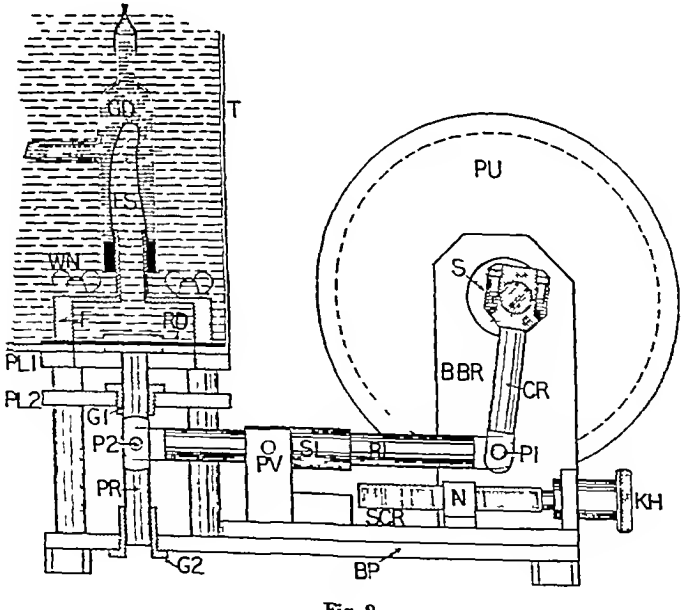
T
GD
PU
S
WN
RD
BBR
CR
PL1
PL2
G1
P2
PV
SL
RL
PI
PR
N
KH
SCR
G2
BP

Fig 2

whole apparatus is not shown in Fig 2 It consists of base plate, *BP*, carrying at one end the crankshaft bearing-bracket, *BBR*, at the other end, a double platform *PL* 1 and *PL* 2, supported on four pillars, of which two only can be seen To the upper platform is attached one end of a tank, *T* The other end is supported by adjustable legs (not shown)

The crankshaft is driven by a pulley, *PU*, and operates two connecting rods, *CR* (only one shown in elevation), by means of separate cranks The lower end of each connecting rod is shaped to fit in a slot cut in the hinder end of a rocking lever, *RL*, and is kept in position by a pin, *P* 1 The forward end of the lever is slotted to form a fork embracing a flattened section of the pump rod, *PR*, to which it is pinned by the pin, *P* 2

The position of the fulcrum of the lever can be adjusted by the following device, either while the pump is in motion or when it is stationary A gunmetal sleeve, *SL*, which is free to slide on the rocking lever, carries at its forward end a square fork, *FK*, lying between two cheek pieces to which it is pivoted at *PV* This pivot is the actual fulcrum of the lever The cheek pieces are attached below to a slide which runs in a dovetailed guide on the base plate The position of the slide is adjusted by means of a screw, *SCR* (which can be rotated by its knurled head, *KH*) and the nut, *N*, attached to the slide When the slide is in its most forward position, the pivot, *PV*, comes exactly opposite the pin, *P* 2, and no motion is imparted to the pump rod When the slide is screwed back as far as possible, the arms of the lever are approximately of equal length and a stroke of about $\frac{5}{8}$ in is given to the pump rod The pump rod, *PR* is vertical and runs in guides, *G* 1 and *G* 2, carried respectively by the lower platform, *PL* 2, and the base plate A rubber diaphragm, *RD*, is attached to its upper end by means of two brass plates, one fixed to the pump rod and the other screwed to the first through the rubber A circular hole, 2 in in diameter, cut in the upper platform and a corresponding hole in the bottom of the tank allow the central portion of the rubber diaphragm to move downward with the pump rod when it is depressed The edge of the diaphragm is clamped between the large end of a thick-walled brass funnel, *F*, and the bottom of the tank by four wing nuts, *WN*, working on bolts which pass through the wall of the funnel, the bottom of the tank and the upper platform, their heads being fixed to the latter

Round the tube of the funnel is fixed the open end of a rubber finger stall, *FS*, and surrounding it, held in position on the tube of the funnel by a rubber bung, is a glass dome, *GD* The funnel and finger stall are filled with water, and the glass dome outside the finger stall with blood or other perfusion fluid When the diaphragm rises, water is forced into

the finger stall, which expands and drives the blood out of the glass dome When the diaphragm falls the reverse process occurs In order to provide for the passage in each direction of the blood, two tubes are blown to the side of the dome at right angles to one another and to the long axis of the dome One of these is connected, as shown in section in Fig 1, to the inlet valve, *IV* and the other to the outlet valve, *OV* The valves themselves are rubber caps each with a transverse cut near its closed end The inlet valve is slipped over the end of a straight glass tube, *ST*, which passes through the tank and out of it by way of a packed gland, *PG*, which forms a water-tight joint between the tube and the tank The valve-chamber is a short section of wide glass tube bent at a right angle and fixed with rubber bungs on the one hand to the straight tube, and on the other to one of the side tubes of the dome The outlet valve is slipped over the other side tube, it is enclosed in a chamber with one wide end attached by a rubber bung to the side tube and the other end narrow The narrow end is connected by rubber tubing with one end of a coil of glass tubing *CO* The coil at its other end passes into a straight tube which emerges from the tank by way of a packed gland A small vertical branch tube comes off each of the outlet valve-chambers, and vertical tubes are blown on to the top of each dome These tubes are used in filling the domes and to allow air to escape When the apparatus is in use, they are closed by short lengths of rubber tubing and clips

Though the figure shows the glass warming-coil attached to the outlet (arterial) side of each pump it will be obvious that it could equally well be attached to the inflow side and the straight tube to the outflow, and this arrangement was, in fact used in the perfusions actually carried out

The tank is filled with water, which is kept at the desired temperature by a long narrow electric glow lamp housed in a brass tube, *LT*, passing across the tank near its bottom and soldered into holes in the sides This lamp can be slid in and out of the tube by hand for rough adjustment of the temperature, or can be connected with a thermostat working a magnetic relay for finer control

Use of the Pump

The pump has not yet been used for its original purpose of producing a complete circulation of the heartless animal. It has been used, however with success for experiments in which the hind quarters of a cat or dog were perfused by one pump and the lungs by the other, defibrinated blood being used Open venous reservoirs were used into which the blood from the abdominal cava and the left auricle respectively discharged, and

from each of which it was sucked into the alternative pump, from "systemic" reservoir into "pulmonary" pump and *vice versa* When the throws of the two pumps are adjusted to the proper relation, the level of the blood is stationary in both venous reservoirs Any change in the relation of the peripheral resistances, usually occasioned by a rise or fall of the systemic resistance, causes one venous reservoir to fill at the expense of the other, and necessitates a new adjustment of the relation between the throws of the pumps if a new equilibrium is to be produced A very satisfactory perfusion can be maintained in this way for many hours

The details of procedure in setting up an experiment of this kind may be given The quantity of blood required to fill the apparatus shown in Figs 1 and 2, including the spaces between finger stalls and glass domes, the valve-chambers, the glass coils and the tubes is about 50 c c Another 50 c c at least is required to provide a small reserve in the venous reservoirs and fill the connecting tubes At least 100 c c of blood, therefore, should be available to fill the system before a perfusion can be begun, and if any but a very small organ is perfused more will be required It is accordingly easy to perfuse a dog's hind limbs and lungs with its own blood, but for perfusion of a cat's limbs and lungs it is necessary to use one cat to provide the first charge of blood for the apparatus and a second to provide a further quantity of blood as well as the organs for perfusion The animals were always bled out under anæsthesia with ether, cats from a cannula tied into the abdominal aorta, dogs from a cannula in a carotid artery The blood was whipped with an indiarubber brush as it was received in a basin, and filtered through muslin, and then through a plug of washed cotton wool By the time that the bleeding was completed, of the animal which was to furnish the organs for perfusion, an assistant had filled the apparatus with the earlier part of the defibrinated and filtered blood The next step was to prepare the lungs for perfusion The animal being killed, the chest was widely opened, and a bulb cannula, with inlet tube and outlet nozzle and side tubes for insertion of a thermometer and for connection with a manometer, was tied by its nozzle into the main pulmonary artery, which had been clamped as near as possible to its bifurcation, opened and washed out with saline A strong ligature was then tied in the auriculo-ventricular groove if this had not been done previously, and the ventricles were usually cut away A wide cannula was tied through a slit in the apex into the appendix of the left auricle A respiratory cannula was tied into the trachea, and this with the attached lungs, heart and cannulæ was removed from the body, and

transferred to the apparatus The arterial cannula being carefully filled with blood was then connected by rubber pressure-tubing with one of the arterial outlets of the pump (say A_1 in Fig 1) The other arterial outlet (A_2) would be connected for the time directly by blood-filled rubber tubing with V_1 The cannula from the left auricle was connected by a rubber tube so as to discharge into the venous reservoir from which, during action of the pump, the blood was sucked by V_2 The connections all being safely made, to the exclusion of bubbles, the artificial respiration-pump was started, and then clips were removed and the circulation-pump set in motion During circulation of the first 50 c c or so through the lungs, the outflowing blood from the left auricle was caught in a dish, whipped and re-filtered, pre-filtered blood from the reserve being added to the reservoir as required during this process Finally all the blood available was returned to the system, in which it now circulated into the pump through V_2, out through A_2, directly from this to V_1 out through A_1 to the pulmonary artery, through the lungs, and from the left auricle back to the reservoir feeding V_2

While the defibrinated blood was thus being circulated through the lungs, cannulæ were being inserted for the systemic perfusion, either into the lower ends of the abdominal aorta and vena cava, for perfusion of both hind limbs, or into the femoral artery and vein for perfusion of one leg In either case, loss of blood through collateral channels had to be excluded by careful mass ligaturing The hind quarters were then detached from the rest of the carcase and transferred to the table adjoining the perfusion-pump, which was now stopped while the attachments were completed The arterial cannula was filled and attached, with exclusion of bubbles, to the pressure-tube leading from A_2, while the blood from the venous cannula was led by a rubber connection to discharge into the reservoir feeding V_1 The pump being started, the first portion of blood issuing from the limb-veins was again caught, whipped, re-filtered, and then returned to the reservoir Connection was now made between the arterial cannulæ and the corresponding manometers and the strokes of the pumps so adjusted that the levels in the two reservoirs remained practically constant

In our experience, as in that of other workers, the use of the lungs for oxygenation preserves the blood in a much more physiological condition than does an artificial oxygenator, such as that devised by Hooker and used by Burn and Dale Not only is the potent vaso-constrictor effect of the freshly defibrinated or heparinised blood removed by the preliminary circulation through the lungs, but the moderate and more

normal vascular tone of the systemic vessels thus produced is maintained during a long perfusion, the vessels long retain their reaction to constrictor and dilator drugs, and œdema of the organs is long postponed

It will be obvious that, where a single pump only, with readily and accurately adjustable throw, is required for any physiological purpose, one pump of the type described above can be used Such a unit, as made by C F Palmer, Ltd , is illustrated in Fig 3 Obviously two or more of

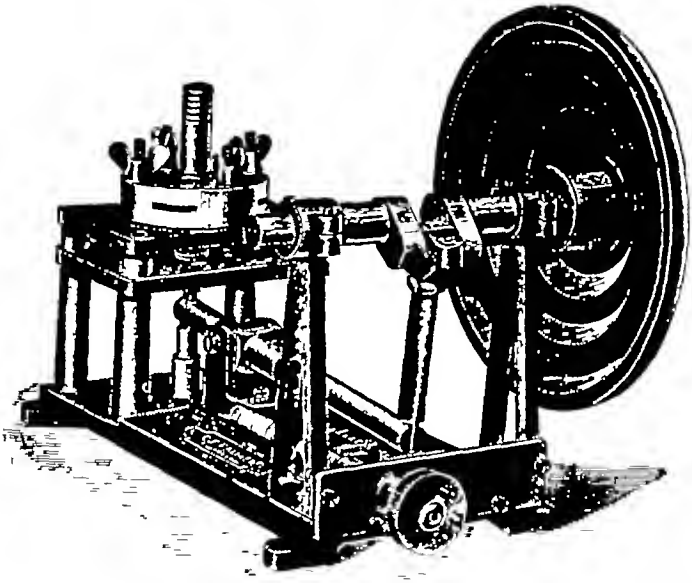

Fig 3

these could be arranged to drive from a common shaft Similarly the thermostat bath, in which the pumps are immersed in the system above described, may for some purposes be omitted or replaced by one more suitable for the particular need We are having one constructed at present in which the tubes from the pumps enter a chamber jacketed with warm water, in which a double perfusion of liver and lungs can be carried out without surface cooling of those organs For the perfusion of the liver, as described by Dr Bodo and Mr Marks in a following paper, the use of the apparatus was further modified by using one pump to raise blood to an overflow reservoir, from which the portal vein was perfused under constant hydrostatic pressure, while a pulsatile pressure was produced

VOL LXIV. No 4

CONTENTS

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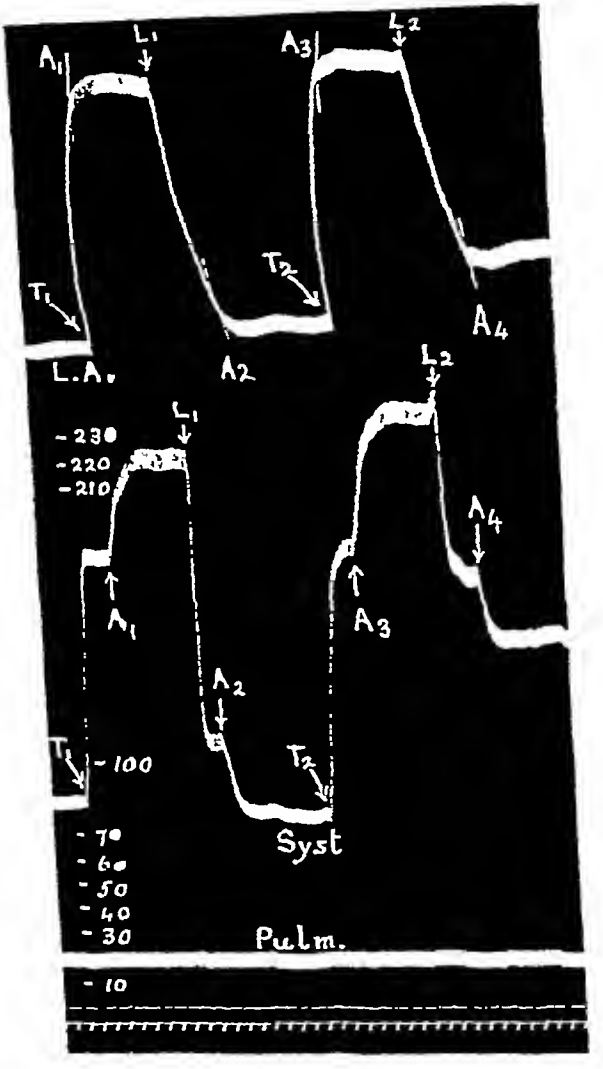

Fig 4 Lines of record, from above downwards Volume of fluid in pulmonary venous reservoir systemic arterial pressure "pulmonary" arterial pressure pressure zero time in 10 sec intervals

THE EFFECT OF THE "HEART-TONICS" AND OTHER DRUGS UPON THE HEART-TONE AND CORONARY CIRCULATION

By RICHARD BODO

(From the Department of Physiology and Biochemistry, University College, London)

THOUGH there exists a considerable literature dealing with the subject of the heart-tonics, it has never clearly shown the uncomplicated effect of these drugs upon the heart The majority of these investigations were made on the frog's heart, but under conditions which are never to be found *in vivo*, in that in these experiments the venous pressure and arterial resistance were identical. Since these conditions do not correspond to the physiological conditions the conclusions drawn from these experiments cannot be entirely convincing Another group of investigators used the mammalian heart as the experimental object and performed their experiments partly on the isolated heart, partly on the whole animal, on hearts left *in situ* Though the isolated heart is quite a useful preparation for some purposes, it is not suitable for the study of the effect of the heart-tonics, because, the heart chambers being more or less empty, the heart performs much less work than *in vivo* the resistance being negligible The experiments performed on the whole animal have shown many very important facts, but in spite of these, they do not show simply the direct effect on the heart, since this might be complicated by the effect produced on the nervous system, which might change the heart rate, with a resulting change in the output Even after exclusion of nervous effects there is another complicating factor to be dealt with practically every heart-tonic having an effect on the vessels as well which might change the venous inflow and ventricular output, thus obscuring the direct effect of the tonic drug on the heart

For the study of the direct effect of a drug upon the heart, Starling's heart-lung preparation seemed to be the most suitable, because in this preparation all the environmental conditions of the heart, viz , temperature, composition, *p*H, volume and pressure of the entering blood, and the opposition to the outflow offered by the arterial resistance, can be exactly

in the hepatic artery by use of the other pump The apparatus can doubtless be adapted in other ways for other special purposes

Fig 4 shows a record of the pressures in the "pulmonary" and "systemic" arterial cannulæ in perfusion through an artificial scheme, the systemic and pulmonary resistances being represented by a high and a low resistance respectively on the two sides of the double circuit The volume recorder, communicating with the air-space above the fluid in the "pulmonary" venous reservoir, equivalent to a stationary left auricle, shows (*LA* in Fig 4) the balance of the circulation through the two widely different resistances produced by adjusting the relation of the two pump-strokes A slight tightening, at T_1, of the clamp producing the "systemic" resistance causes a rise of systemic arterial pressure from about 80 to 180 mm., and an increase of the volume of fluid in the pulmonary venous reservoir, at the expense of that in the systemic reservoir A small adjustment of the pump at A_1 restores the balance at the new relation of resistances, but the increase of stroke required to effect this causes the systemic pressure to rise further to about 220 mm At L_1 the clamp producing the systemic resistance is slightly relaxed, producing a fall of systemic arterial pressure, and a loss of fluid from the pulmonary venous reservoir To balance this loss, the stroke of the systemic pump must again be reduced at A_2, producing a further fall in the systemic pressure The whole cycle is then repeated at T_2, A_3, L_2 and A_4 It will be seen that the apparatus is made to produce, after a delay allowing the disturbance of equilibrium to become manifest, that adjustment of the work done by the pump to the resistance encountered by the outflow, which is necessary to restore equality of output from both pumps This is, of course, the adjustment which the living ventricle makes immediately and with great precision, and failing which any increase of systemic resistance would produce rapid dilatation of the left auricle, and back pressure on the lungs Apart from its use in carrying out experimental perfusions, and studying the effects of vascular reactions uncomplicated by this automatic adjustment of the living heart, the apparatus might have some value for purposes of demonstration, enabling the nature of this adjustment, and its necessity, to be shown with great clearness and precision

Summary

A double perfusion-pump is described, by which any part of the systemic vascular system and the pulmonary system may be perfused in continuity, and the outputs adjusted in relation to the varying resistances while the pump is in motion

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controlled, changed within very wide limits, or kept unaltered during the whole of the experiment In this preparation the frequency of the beat depends only on the temperature of the pace-maker, provided that the *p*H of the blood is constant, and as has been shown in previous work, the hydrogen ion concentration of the blood, its CO_2 and lactic acid content do not change appreciably during the course of an experiment after the first quarter of an hour So that, by keeping the temperature of the entering blood constant, we can be assured of a regular rhythm throughout the whole of the experiment

Further, it is quite easy to maintain the work of the heart constant, or to alter it within wide limits by changing the inflow or the arterial resistance In this preparation the direct effect of a drug upon the heart ought to be seen quite clearly Hitherto, however, no experiments have been performed on the effects of the heart-tonics on the heart-lung preparation, with the exception of one investigation by Buijsma and Roessingh(1), who used the heart-lung preparation from the cat in order to investigate the effect of Strophanthin It seemed worth while, accordingly, to investigate more fully the effect of the heart-tonics upon the heart under these conditions

In the first place clear definitions must be given of the meaning in the present paper of the terms "heart-tone" and "heart-tonics," since the term "tone" has been employed by many investigators in dealing with the heart, both in physiological and pathological literature, with a different implication Here it will be used as Starling defined it, in one of his earlier papers(2), as "synonymous with physiological condition or fitness of the muscle fibre," and more recently and more exactly(3) as "the mechanical efficiency of the muscle fibre, *i e* the relation of the mechanical energy to the total energy liberated" It was found by Starling and Visscher(3) that the oxygen consumption of the heart—which served as a measure in their experiments of the total energy set free in the heart during its activity—provided that the chemical and temperature conditions were maintained constant, was determined by the initial length of its muscular fibres, *i e* by its diastolic volume That means that, if the same work is done by two hearts, in one case with a small diastolic volume and in the other with a large diastolic volume, the mechanical efficiency of the former is greater, the tone of the former is better than that of the latter This enables us to measure the heart-tone by measuring the diastolic volume, provided that the work performed by the heart is maintained constant during the whole of an experiment It may accordingly be expected of a drug which is described as heart-tonic,

that it will enable the heart to perform the same work as before with a smaller volume

Methods All the experiments have been carried out on dogs, the heart-lung preparation was made in the usual way, as described by Starling(4) and his fellow workers from this Laboratory The heart rate was maintained constant by keeping the temperature of the heart constant The temperature was measured in the blood entering the heart The rate of the beat was recorded on a kymograph by a tambour, which was connected by air transmission with a small piston recorder, the piston of which was pulled by means of a thread by the contracting auricle The output of the heart and the arterial pressure were maintained constant by keeping the venous inflow and the arterial resistance constant. The output of the left ventricle was determined by measuring the amount of blood flowing out on the venous side of the resistance This did not include the coronary flow, in order to determine the latter a Morawitz cannula was introduced into the coronary sinus According to Evans and Starling(5) and Markwalder and Starling(6) this drains only three fifths of the coronary blood. The arterial pressure was measured by connecting the cannula in the innominate artery by means of a side branch with a mercurial manometer, the oscillations of which were recorded on the kymograph. The amount of blood entering the heart was adjusted to the size of the heart, and it varied from 300–600 c.c. per min. The arterial resistance was adjusted to produce an arterial pressure of 100 mm. Hg Under these conditions the volume of the heart lung preparation remains for a time steady, but later, as the heart tires, it dilates continuously The drug was administered into the venous blood reservoir in some of the experiments this was done at a time while the heart volume was still normal, but in the majority of the experiments when the heart had become already tired and dilated, since it was thought that the conditions of the dilated heart would correspond more closely with those found in the pathological state In order to produce the heart dilation quickly at the beginning of an experiment either the venous inflow or the arterial resistance or both were increased. In order to measure the volume of the heart a Henderson cardiometer was employed, fitted with a special rubber sleeve of very thin material, which lay snugly against the heart The cardiometer was connected by air transmission with Palmer's large piston recorder, the volume change of which was recorded on a kymograph. Special care was taken to make an absolute air tight seal around the ventricles without compressing the atrio ventricular ring To control the pressure in the right auricle—especially when fixing the cardiometer on the heart—a cannula was introduced into the inferior vena cava which was connected with a saline manometer

Among the heart-tonics, the effects of the following have been investigated Digitalis, Caffeine, Camphor and Strychnine

Experiments on the Heart-Tone

Digitalis Of the Digitalis group the effects investigated were those of *g*-Strophanthin, of Digitalis tincture and of the Digitalis infusion In the first experiments *g*-Strophanthin was tested, and it was used in the same dose as was employed by Bijlsma and Roessingh(1) on the cat's heart, namely, 0 03 mgm *g*-Strophanthin per 100 c c blood In our experiments this dose, however, very soon caused a toxic effect It was necessary to perform a great number of experiments before the proper dose was found, in the earlier experiments it was either given in too small

a dose, which had no effect, or in too large a dose, which led very quickly to poisoning of the heart When used in the right dose, which was found in our experiments to be 0 0025 mgm *g*-Strophanthin per 100 c c of blood, it improves the function of the heart In most experiments the improvement is a real tonic effect, the heart volume becoming smaller, though the work performed by the heart is the same as before, the output, arterial resistance and heart rate being the same If the improving effect is most marked (optimal), the heart comes back to its original volume (Fig 1) That the output, even at that smaller heart volume, remains the

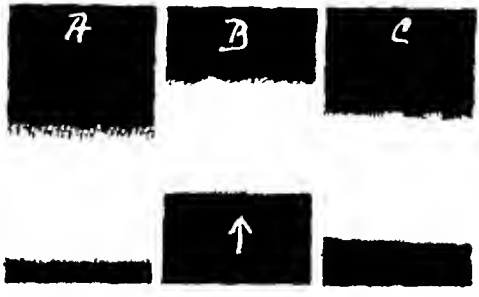

Fig 1 Strophanthin.

A = heart normal Temp 36 0° C. Heart rate = 138 Outflow = 550 c c. per min. B_P = 100 mm Hg V_P = 9 5 cm. H_2O Heart's weight = 88 gm. Amount of blood = 1000 c.c *B* = heart dilating At † 0 025 mg *g*-Strophanthin (0 0025 mg per 100 c.c of blood) was added. *C* = 30 min. later Heart volume diminishing, tonic effect

same as before, can be observed not only by measuring the amount of blood flowing out on the venous side of the resistance, but it can also be seen from the cardiometer tracing, the difference between the systolic and diastolic volumes, which shows the output, remaining unaltered (Fig 1)

Sometimes the improvement is only a prevention of further dilatation of the heart, and sometimes there is no improvement at all, possibly in such cases the drug was given too late so that the heart could not react any more Whatever improvement occurred was not apparent immediately after the administration of the drug, it was manifest only about 15–30 min later, but it was lasting Strophanthin in the dose used here did not change the heart rate

Digitalis tincture produced almost the same effect (Fig 2), and the effect appeared about the same time after the administration

It seemed possible that quicker and more regularly definite results

might be obtained by using a Digitalis preparation not containing alcohol like the two already discussed, since it is known from Sulzer's work(7)

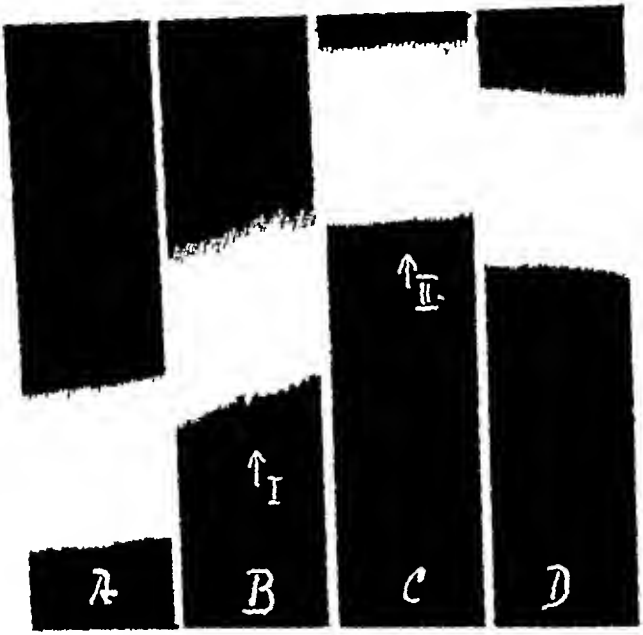

Fig 2. Digitalis tincture.

A = heart slightly dilating Temp 37 0° C. Heart rate = 150 Outflow = 600 c.c. per min. B.P = 130 mm. Hg V P = 9 5 cm. Heart's weight = 73 gm. *B* = well marked dilation. At ↑I 0 5 c.c. Digitalis tincture was added. *C* = 5 min. later Heart still further dilating At ↑II 1 0 c c. Digitalis tincture was added. *D* = 20 min. later, tonic effect

that alcohol causes a heart dilatation in the heart-lung preparation, and it seemed conceivable that the dilating effect of alcohol might weaken or antagonise the tonic effect of Digitalis In order to exclude the complicating effect of alcohol, an infusion of Digitalis leaves (in the concentration of 1 100) was prepared and used for the experiments, the tonic effect obtained in this way was more marked, the volume decreased in every experiment to normal, but the effect was again produced only after 15–20 min (Fig 3)

The tonic effect caused by all these preparations of Digitalis though delayed in onset was lasting, except when the dose was too large, so that the tonic effect was followed very soon by the toxic effect

Caffeine In contrast to the effect of Digitalis, which, as shown above,

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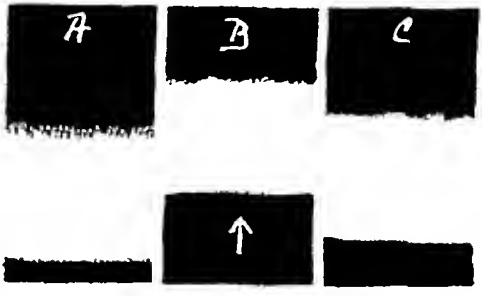

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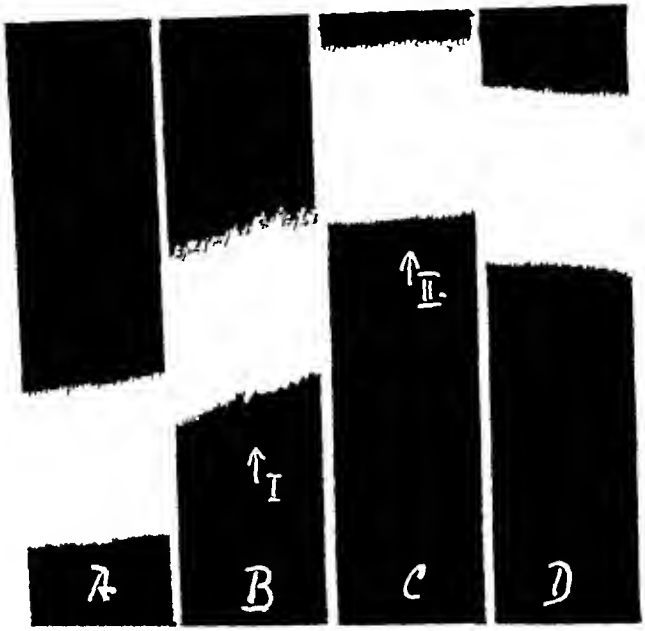

Fig 2. Digitalis tincture.

A=heart slightly dilating Temp 37 0° C. Heart rate=150 Outflow=600 c.c per min. B P =130 mm. Hg V P =9 5 cm. Heart's weight=73 gm. *B*=well marked dilation. At †I 0·5 c.c. Digitalis tincture was added. *C*=5 min. later Heart still further dilating At †II 1 0 c c Digitalis tincture was added. *D*=20 min. later, tonic effect

that alcohol causes a heart dilatation in the heart-lung preparation, and it seemed conceivable that the dilating effect of alcohol might weaken or antagonise the tonic effect of Digitalis In order to exclude the complicating effect of alcohol, an infusion of Digitalis leaves (in the concentration of 1 100) was prepared and used for the experiments, the tonic effect obtained in this way was more marked, the volume decreased in every experiment to normal, but the effect was again produced only after 15–20 min (Fig 3)

The tonic effect caused by all these preparations of Digitalis though delayed in onset was lasting, except when the dose was too large, so that the tonic effect was followed very soon by the toxic effect

Caffeine In contrast to the effect of Digitalis, which, as shown above,

is delayed in onset but lasting in character, the tonic effect of Caffeine is rapid in onset but evanescent (Fig 4) The Caffeine was administered as

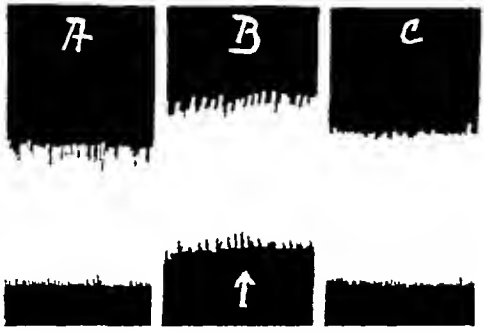

Fig 3 Digitalis infusion.

A = heart normal Temp 37·0° C Heart rate = 145 Outflow = 571 c.c per min. B.P in the beginning = 110 mm. Hg, later = 130 mm. Hg V P = 8 0 cm H_2O Heart's weight = 82 gm. *B* = heart dilating At ↑ 10 0 c.c Digitalis infusion (1 100) were added. *C* = 20 min. later, tonic effect.

the double benzoate of caffeine and sodium, in such a dose (0 05 to 0 1 gm of caffeine reckoned as base) that the rate of the heart was not affected When the effect of the Caffeine is over the heart resumes its slow dilatation,

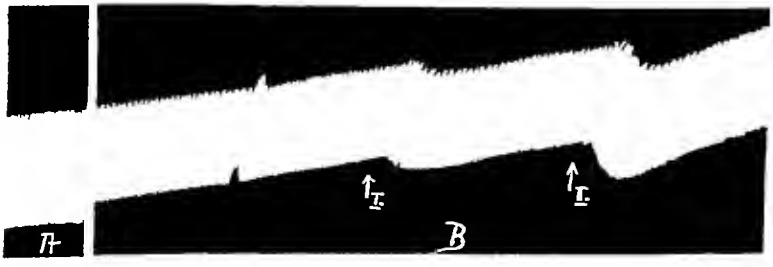

Fig 4 Caffeine

A = heart slightly dilating Temp 37 0° C Heart rate = 153 Outflow = 500 c.c per min. B.P = 140 mm. Hg V P = 9 5 c c Heart s weight = 133 gm. *B* = Heart further dilating At ↑I 0 05 gm. Caffeine was added, transitory tonic effect At ↑II 0 10 gm Caffeine was added, transitory tonic effect

and the effect of Caffeine can be repeated several times, since the tonic effect does not give way to toxic action, as in the case of Digitalis

Camphor To our surprise and in contrast to the tonic effects of Digitalis and Caffeine, Camphor caused dilatation of the heart In the

earliest experiments the alcoholic solution of Camphor was used (0 10 gm.) and produced in every case a well-marked heart dilatation (Fig 5) The

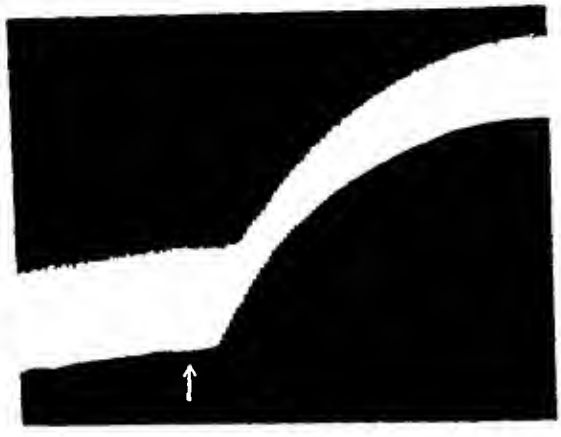

Fig 5 Camphor (dissolved in Alcohol Saline)

Temp. 37 0° C Heart rate = 158. Outflow = 400 c c. per min. B.P = 105 mm. Hg V.P = 8 0 cm. Heart's weight = 81 gm. Heart dilating At ↑ 0 10 gm. Camphor dissolved in Alcohol-Saline was added, caused a well-marked dilatation.

result obtained seemed to be in contradiction to the generally accepted medical opinion concerning Camphor, and therefore the possibility that the solvent alcohol might be responsible for the dilatation of the heart had first to be excluded Camphor oil could not be used for our purpose and the Aqua Camphorata U S P was therefore used as the only suitable preparation. This was prepared according to the prescription of the U S P , 0 8 gm Camphor being triturated with 0 8 c c of 95 p c Alcohol in a mortar, 1 5 gm Purified Talc being then added and the trituration continued until the Alcohol evaporated, 100 c c recently boiled distilled H_2O being finally added The mixture was repeatedly filtered until the Camphor Water became perfectly clear The Camphor content of Camphor Water prepared in this way was not exactly known, because a great amount of the Camphor was retained on the Talc and probably only a very small part went through the filter, 10–20–30 c c of this solution were used for the experiments (that is, the average therapeutic dose of this preparation according to the U S P), and these doses produced in every case a very marked dilatation of the heart In order to make these results quite convincing, there were two factors still to be excluded Firstly, the large amount of fluid used might be responsible for the dilating effect

obtained (change in the viscosity of the blood?), and secondly—since the Aqua Camphorata still contains traces of Alcohol—the Alcohol present might be the responsible factor Control experiments were performed for this purpose 20–30 c c Saline were added to the venous reservoir to control the volume-effect, and an Alcohol-Saline solution was prepared in the same way as the Aqua Camphorata U S P , containing all the constituents of the Aqua Camphorata except the Camphor, and 20–30 c c of this solution were similarly added These experiments, however, produced only negative results, neither the 20–30 c c Saline nor the 20–30 c c Alcohol-Saline solution produced any dilatation of the heart (Fig 6)

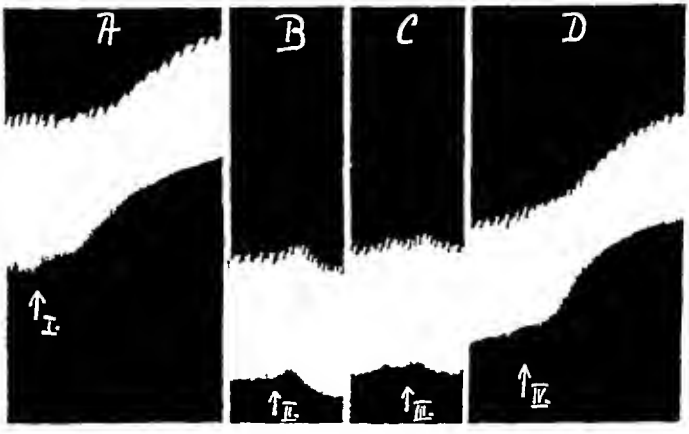

Fig 6 Aqua Camphorata U S P Saline Alcohol-Saline

Temp 37 0° C. Heart rate 138 per min Outflow = 430 c.c. per min. B.P = 110 mm Hg V P = 7 0 cm. H_2O Heart's weight = 101 gm. *A* = Heart normal, at ↑I 20 0 c.c. Aqua Camphorata U S P added. *B* At ↑II 30·0 c.c of Saline added. *C* At ↑III 20 0 c.c. Alcohol Saline (0 8 100) added. *D* At ↑IV 20 0 c.c. Aqua Camphorata U S P added.

It is accordingly proved that the dilatation of the heart following administration of Camphor is a genuine effect of that substance Camphor in the dose used did not alter the heart-rate

Strychnine Finally Strychnine was tested in this preparation, because it has been largely used by clinicians on the assumption that this drug has a tonic effect on the human heart In the heart-lung preparation from the dog no such effect has been seen (Fig 7)

EFFECTS OF THE ABOVE DRUGS ON THE CORONARY CIRCULATION

It seemed worth while to investigate the effect of these drugs upon the coronary circulation because there was the possibility that the tonic

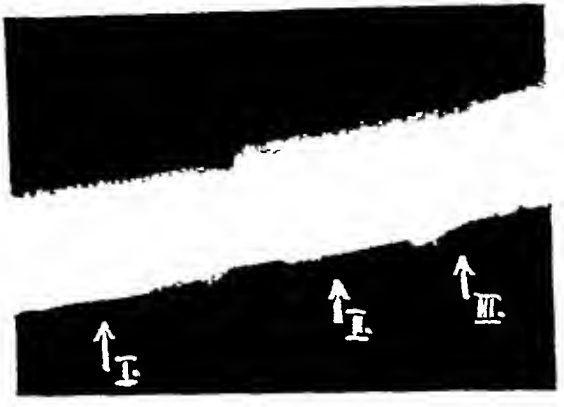

Fig 7 Strychnine

Temp 37·0 C Heart rate 142 per mm. Outflow = 413 c.c. per min. B.P = 110 mm. Hg V.P = 8 cm. H_2O Heart's weight = 82 gm. At I 0·0002 gm. Strychnine sulph. added. At II 0·0004 gm. Strychnine sulph. added. At III 0·0010 gm. Strychnine sulph. added.

effect was only a consequence of the effect produced on the coronary vessels It was quite conceivable that a drug might produce its tonic effect by dilating the coronary vessels and thereby improving the blood flow through the heart muscle On the other hand there was the possibility that the sequence might be the opposite of this the primary effect being the action on the heart muscle fibres a tonic effect causing a compression of the coronary vessels or a dilating action on the muscle producing a passive widening of the coronary vessels

The experiments performed for this purpose (Tables I II III IV) show that the Digitalis group (Strophanthin Digitalis tincture) Caffeine and Camphor all dilate the coronary vessels The increase in the coronary flow by Strophanthin and Digitalis tincture was only a moderate one and it did not appear immediately, Caffeine increased the coronary flow much more and the effect was instantly noticeable Camphor produced only a slight increase in the coronary flow

TABLE IV

Temp. 36·0° C Heart rate = 138 per min. B.P = 110 mm. Hg

Time	System output c.c. per min	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
1 08	462	36	60	
1 09	—	—	—	1·0 c.c Aqua Camphorata U.S P
1 10	462	36	60	
1 13	462	39	65	
1 14	—	—	—	5·0 c.c. Aqua Camphorata U S P
1 16	—	42	70	
1 18	—	44	73	
1.19	375	47	78	
1 20	—	—	—	20·0 c.c Aqua Camphorata U.S P
1.22	364	60	100	
1.24	—	63	105	
1.26	—	63	105	
1.27	—	—	—	25 0 c.c. Aqua Camphorata U S P
1 28	308	78	130	
1 31	—	81	135	
1 32	—	—	—	25 0 c.c. Aqua Camphorata U S P
1.34	353	90	150	
1 37	—	93	155	

EFFECTS OF CERTAIN OTHER DRUGS

In order to obtain yet further evidence as to a possible relationship between effects on the heart muscle, on the one hand, and on the coronary circulation on the other it was thought desirable to examine by the same methods certain other substances which had no reputation as heart-tonics, but were known or suspected to have an action on the coronary vessels

Nitrites Though Lauder-Brunton(8) introduced Amyl nitrite in therapeutics in 1867, its vaso-dilator effect on the coronary arteries was only later demonstrated by Cow(9) and Pal(10) on isolated vessel strips, and by Schloss(11) and Loeb(12) on the coronary circulation Our purpose is to determine whether it would have the same effect in the denervated heart-lung preparation

As can be seen in Tables V and VI both Amyl nitrite and Sodium nitrite increased the coronary flow also in this preparation, and the effect appears very rapidly and can be repeated many times With regard to their action on the heart-tone there is a difference between them Amyl nitrite having no effect at all and Sodium nitrite, in larger doses, causing a heart dilatation (Figs 8 and 9)

TABLE I.

Temp. 37 0° C Heart rate = 150 per min. B.P = 100 mm. Hg

Time	System output c.c per min.	Coronary sinus output c c per min.	Total coronary* flow (calc) c.c per min.	Addition of
1 00	430	51	85	
1 10	—	54	90	
1 15	—	54	90	
1 16	—	—	—	0 1 c c *g*-Strophanthin
1 18	430	51	85	
1 20	—	51	85	
1 21	—	—	—	0 2 c.c *g*-Strophanthin
1 23	430	60	100	
1 26	—	60	100	
1 35	—	69	115	

* The total coronary flow figures were obtained from the coronary sinus output by multiplying them by 5/3

TABLE II

Temp 37 0° C. Heart rate = 150 per min. B.P = 100 mm Hg

Time	System output c.c. per min.	Coronary sinus output c c. per min	Total coronary flow (calc.) c.c. per min	Addition of
3 08	550	32	53	
3 13	—	32	53	
3 14	—	—	—	0 5 c c. Digitalis tincture
3 16	—	32	53	
3 19	550	32	53	
3.25	—	32	53	
3.27	—	—	—	0 5 c.c Digitalis tincture
3 29	—	32	53	
3 33	—	—	—	1 0 c.c Digitalis tincture
3 38	—	—	—	1 0 c c. Digitalis tincture
3 39	550	40	66	
3 40	—	—	—	6 0 c.c. Digitalis tincture
3 41	—	56	93	
3 44	500	71	118	
3 47	—	72	120	
3 48	—	—	—	Toxic effect

TABLE III.

Temp 37 0° C Heart rate = 162 per min. B P = 110 mm. Hg

Time	System output c.c. per min.	Coronary sinus output c.c per min.	Total coronary flow (calc.) c.c. per min.	Addition of
1.55	375	41	68	
2.05	—	43	72	
2 11	—	43	72	
2.12	—	—	—	0 10 gm. Caffeine
2 13	—	99	165	
2 14	—	108	180	
2.21	261	111	185	
2 22	—	—	—	0 10 gm. Caffeine
2.24	—	135	225	
2 27	240	132	220	

TABLE IV

Temp. 36·0° C Heart rate = 138 per min. B.P = 110 mm. Hg

Time	System output c.c. per min	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
1 08	462	36	60	
1 09	—	—	—	1·0 c.c Aqua Camphorata U.S P
1 10	462	36	60	
1 13	462	39	65	
1 14	—	—	—	5·0 c.c. Aqua Camphorata U S P
1 16	—	42	70	
1 18	—	44	73	
1 19	375	47	78	
1.20	—	—	—	20·0 c.c. Aqua Camphorata U.S P
1 22	364	60	100	
1.24	—	63	105	
1.26	—	63	105	
1.27	—	—	—	25 0 c.c. Aqua Camphorata U.S.P
1.28	308	78	130	
1 31	—	81	135	
1 32	—	—	—	25 0 c.c. Aqua Camphorata U S P
1 34	353	90	150	
1 37	—	93	155	

EFFECTS OF CERTAIN OTHER DRUGS

In order to obtain yet further evidence as to a possible relationship between effects on the heart muscle, on the one hand, and on the coronary circulation on the other it was thought desirable to examine by the same methods certain other substances which had no reputation as heart-tonics, but were known or suspected to have an action on the coronary vessels

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As can be seen in Tables V *and* VI both Amyl nitrite and Sodium nitrite increased the coronary flow also in this preparation and the effect appears very rapidly and can be repeated many times With regard to their action on the heart tone there is a difference between them Amyl nitrite having no effect at all and Sodium nitrite, in larger doses, causing a heart dilatation (Figs 8 and 9)

TABLE V

Temp 37 0° C Heart rate = 142 per min. B.P = 100 mm. Hg

Time	System output c c. per min.	Coronary sinus output c c. per min.	Total coronary flow (calc.) c c per min.	
1 55	444	36	60	
2 00	—	36	60	
2 05	—	36	60	
2 08	469	—	—	
2 11	—	36	60	
2.12	—	—	—	3 minims Amyl nitrite mixed in the inspired air
2 13	—	84	140	
2 15	—	60	100	
2 17	444	45	75	
2 23	—	33	55	
2 24	—	—	—	
2 24	—	96	160	3 minims Amyl nitrite
2.25	—	90	150	
2 26	—	51	85	
2 27	—	60	100	
2 28	429	54	90	
2 31	—	45	75	
2 32	—	45	75	
2 32	—	—	—	3 minims Amyl nitrite
2.33	—	87	145	
2 34	—	66	110	
2 35	—	60	100	
2 35	—	—	—	3 minims Amyl nitrite
2.36	—	72	120	
2 37	—	111	185	
2.38	340	111	185	
2 39	—	105	175	
2 40	—	96	160	
2 41	—	87	145	
2 42	—	78	130	
2 43	—	72	120	
2 44	—	66	110	
		72	120	

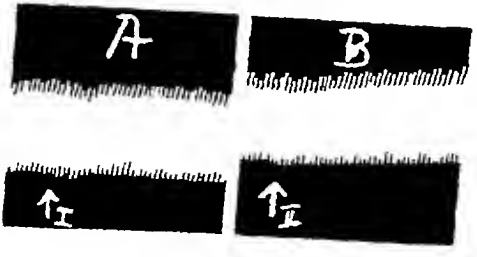

Fig 8 Amyl nitrite

A = Heart normal. Temp 36 0° C. Heart rate = 142 per min. Outflow = 444 c c. per min. B.P = 130 mm Hg V P = 7 0 cm H_2O Heart's weight = 79 gm. At ↑I 3 minims Amyl nitrite mixed with the inspired air *B* = Heart slightly dilating At ↑II 3 minims Amyl nitrite injected into the venous blood close to the heart

TABLE VI.

Temp 37·0° C Heart rate = 148 per min. B.P = 110 mm. Hg
Heart's weight = 130·0 gm.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c per min.	Addition of
4.36	444	35	58	
4.41	—	35	58	
4.46	—	35	58	
4.47	—	—	—	0·05 gm Sodium nitrite
4.49	—	41	68	
4.53	—	45	75	
4 55	444	45	75	
4.56	—	—	—	0 10 gm. Sodium nitrite
4.58	—	54	90	
5 00	—	66	110	
5 01	—	—	—	0 15 gm. Sodium nitrite
5 02	—	71	118	
5 05	—	90	150	
5 06	—	102	170	
5 08	345	114	190	
5 10	—	—	—	0·15 gm. Sodium nitrite
5.13	250	162	270	
5 15	—	180	300	
5 17	—	195	325	
5 19	—	213	355	
5 23	—	240	400	

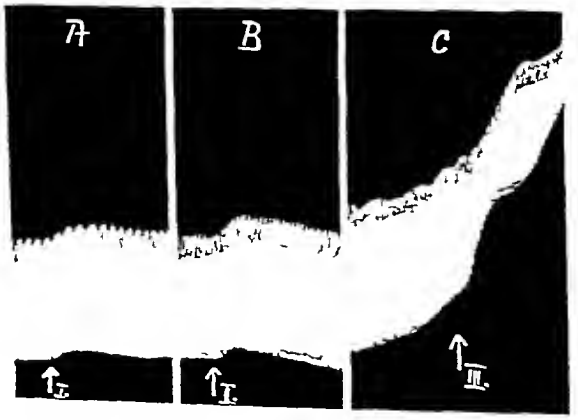

Fig 9 Sodium nitrite

A = Heart normal. Temp. 37·0 C Heart rate = 132 per min. Outflow = 429 c.c. per min. B.P = 100 mm. Hg V P = 6·0 cm. H_2O Heart's weight = 89 gm. At I 0·10 gm Sodium nitrite was added. *B* At II 0·15 gm. Sodium nitrite was added. *C* At III 0·30 gm. Sodium nitrite was added, heart dilating

TABLE V

Temp 37 0° C Heart rate = 142 per min. B P = 100 mm. Hg

Time	System output c c. per min.	Coronary sinus output c c per min.	Total coronary flow (calc) c.c. per min.	Addition of
1 55	444	36	60	
2 00	—	36	60	
2.05	—	36	60	
2.08	469	—	—	
2 11	—	36	60	
2.12	—	—	—	3 minims Amyl nitrite mixed in the inspired air
2 13	—	84	140	
2 15	—	60	100	
2 17	444	45	75	
2 23	—	33	55	
2 24	—	—	—	3 minims Amyl nitrite
2 24	—	96	160	
2 25	—	90	150	
2 26	—	51	85	
	—	60	100	
2 27	—	54	90	
2 28	429	45	75	
2.31	—	45	75	
2 32	—	—	—	3 minims Amyl nitrite
2.32	—	87	145	
2 33	—	66	110	
2 34	—	60	100	
2 35	—	—	—	3 minims Amyl nitrite
2 35	—	72	120	
2.36	—	111	185	
2 37	—	111	185	
2.38	340	105	175	
2 39	—	96	160	
2 40	—	87	145	
2 41	—	78	130	
2.42	—	72	120	
2 43	—	66	110	
2 44	—	72	120	

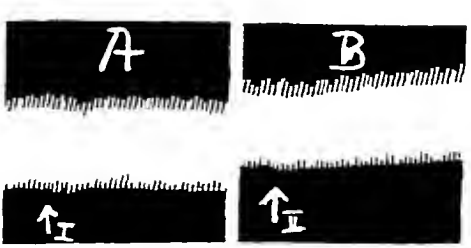

Fig 8 Amyl nitrite.

A = Heart normal. Temp 36 0° C. Heart rate = 142 per min. Outflow = 444 c c. per min. B P = 130 mm. Hg V.P = 7 0 cm H.O Heart s weight = 79 gm At ↑I 3 minims Amyl nitrite mixed with the inspired air *B* = Heart slightly dilating At ↑II 3 minims Amyl nitrite injected into the venous blood close to the heart

TABLE VI.

Temp 37 0° C Heart rate = 148 per min. B P = 110 mm Hg
Heart's weight = 130 0 gm

Time	System output c c per min.	Coronary sinus output c.c. per min	Total coronary flow (calc.) c c per min.	Addition of
4.36	444	35	58	
4 41	—	35	58	
4.46	—	35	58	
4 47	—	—	—	0 05 gm Sodium nitrite
4.49	—	41	68	
4 53	—	45	75	
4 55	444	45	75	
4 56	—	—	—	0 10 gm Sodium nitrite
4 58	—	54	90	
5 00	—	66	110	
5 01	—	—	—	0 15 gm Sodium nitrite
5 02	—	71	118	
5 05	—	90	150	
5 06	—	102	170	
5 08	345	114	190	
5 10	—	—	—	0 15 gm Sodium nitrite
5 13	250	162	270	
5 15	—	180	300	
5 17	—	195	325	
5 19	—	213	355	
5 23	—	240	400	

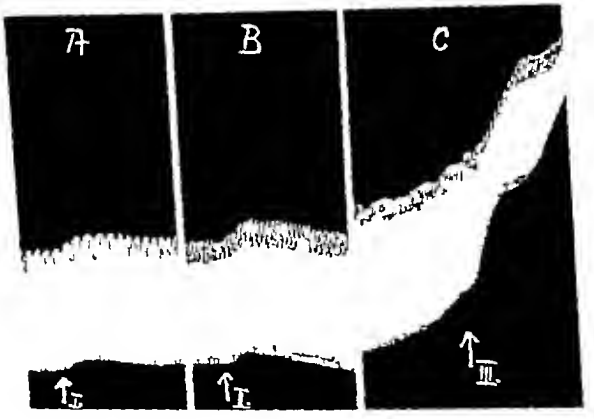

Fig 9 Sodium nitrite

A = Heart normal Temp 37 0° C Heart rate = 132 per min. Outflow = 420 c.c. per min. B P = 100 mm. Hg V P = 6 0 cm H_2O Heart's weight = 89 gm At ↑I 0 10 gm Sodium nitrite was added. *B* At ↑II 0 15 gm Sodium nitrite was added. *C* At ↑III 0·50 gm Sodium nitrite was added, heart dilating

Insulin It was recently shown by Visscher and Müller(23) from this Laboratory that Insulin produces a tonic effect, that is to say that, provided the heart rate, the venous inflow and the arterial resistance are maintained constant, the work performed by the heart, and the output remain the same, but the heart volume is diminished under its action. This effect, which lasts very long, was obtained in our experiments with as little as five units of Insulin and could not be repeated, a second dose being always ineffective (Fig 10) The effect of Insulin was tested on the

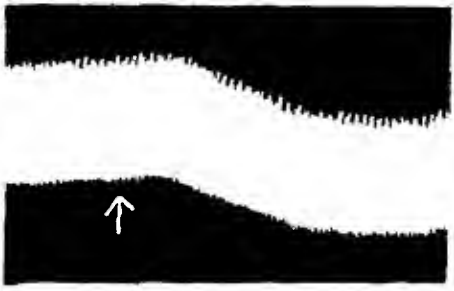

Fig 10 Insulin.

Temp 37 0° C. Heart rate = 150 per min. Outflow = 522 c c. per min. B P = 110 mm. Hg V.P = 8 0 cm. H_2O Heart's weight = 86 gm. Before the administration of Insulin 1 0 gm Dextrose was added. At † 10 units of Insulin were added. Heart volume diminishing, tonic effect

TABLE VII.

Temp 37 0° C Heart rate 152 per min. B.P = 110 mm. Hg

Time	System output c.c. per min.	Coronary sinus output c c. per min.	Total coronary flow (calc.) c.c. per min.	Addition of
1 40	444	75	125	
1 45	—	78	130	
1.50	—	78	130	
1 51	—	—	—	1 0 gm. Dextrose
1 55	—	90	150	
1 56	—	—	—	5 units of Insulin
2 00	444	83	138	
2.05	—	93	155	
2 10	444	93	155	
2 13	—	—	—	10 units of Insulin
2 18	—	66	110	
2.19	—	60	100	
2 23	—	60	100	
2.28	—	66	110	
2.30	—	—	—	10 units of Insulin
2 34	—	54	90	
2 39	—	51	85	
2 46	—	54	90	

coronary circulation (Table VII), and it was found that a small dose (five units), which was sufficient to produce a tonic effect, had either no influence on the coronary circulation or caused a slight transitory constriction, but that a larger dose (ten units) constricted the coronary vessels, diminishing the coronary flow

Pituitrin Pituitrin, which has a vaso-constrictor effect on the vessels in general (Oliver and Schäfer(13)), diminished also the coronary flow in Dale's(14) experiment on the isolated heart This latter effect was observed in this preparation as well, but could be produced only once, not more, even with a larger dose (Table VIII) Its effect on the heart

TABLE VIII.

Temp 37 0° C Heart rate = 150 per min. B P = 110 mm Hg
Heart's weight = 89 0 gm.

Time	System output c.c. per min.	Coronary sinus output c.c per min.	Total coronary flow (calc.) c c. per min	Addition of
1 15	444	20	33	
1 25	—	21	35	
1 34	444	23	38	
1 35	—	—	—	0 1 c c. Pituitrin
1 37	444	17	28	
1 38	—	14	23	
1 39	—	14	23	
1 40	—	14	23	
1 43	444	15	25	
1 47	—	17	28	
1 53	444	22	37	
1 54	—	—	—	0 2 c.c. Pituitrin
1 55	—	21	35	
1 57	—	18	30	
1 59	462	23	38	
2 01	—	24	40	
2 02	—	—	—	0 5 c c Pituitrin
2 03	—	24	40	
2 04	462	24	40	
2.06	—	28	47	
2 08	—	30	50	
2.10	—	30	50	
2.12	462	32	53	

volume was also observed and it was found that it dilated the heart immediately after its administration in a very high degree, but that this dilatation was of transitory character and could also be observed only once or, in some of our experiments, twice, but no more, if a second dilatation occurred it was never so marked as the first (Fig 11)

As in our experiments the volumes of both ventricles are recorded together and cannot be separated, it was conceivable that in the observed heart dilatation after the administration of Pituitrin the right ventricle played a greater part, for which an increase in the resistance of the

pulmonary system, caused either by the direct constriction of the vessels or by the compression of these vessels by the constricted bronchioli,

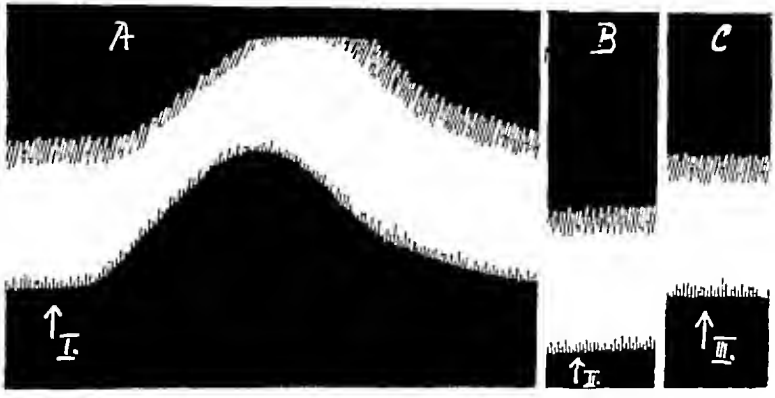

Fig 11. Pituitrin.

A Heart normal. Temp 37 0° C Heart rate = 148 per min. Outflow = 492 c c per min. B P = 130 mm Hg V P = 8 0 cm H_2O Heart's weight = 109 gm. At ↑ I 0 1 c c. Pituitrin was added. Transitory dilating effect *B* At ↑ II 0 2 c.c. Pituitrin was added. Again normal. *C* At ↑ III 1 0 c c Pituitrin was added.

might be responsible In order to investigate this possibility, the pressure was measured in the pulmonary system by inserting a cannula in one of the branches of the pulmonary artery and connecting it with a saline manometer In Table IX, it can be seen that there occurred practically

TABLE IX.

Temp 37 0° C Heart rate = 158 per min. B.P = 130 mm. Hg
Heart's weight = 143 0 gm.

Time	System output c.c. per min.	Venous pressure cm. H_2O	Pulmonary pressure cm. H_2O	Addition of
1 25	500	10 5	14–16	
1 27	—	—	—	0 1 c.c. Pituitrin
1 31	500	13 5	15–17	Heart dilated
1 45	500	10 5	14–16	Heart volume again normal
1 48	—	—	—	1·0 c.c. Pituitrin
1 52	500	11	15–17	No effect

no change in the pulmonary pressure during the whole of the experiment, so that this could not be the cause of the heart dilatation The concordance in the appearance and duration of the phenomena observed, both in the heart dilatation and diminished coronary flow, is so striking, that it makes it highly probable that in the case of Pituitrin they are in causal

relationship, the constriction of the coronary flow being the primary effect

Quinidine As it has been stated by v Frey(15) and by Lewis and his co-workers(16) that Quinidine is more effective than Quinine, the alkaloid originally used by Wenckebach(17) in restoring the normal rhythm in cases of clinical fibrillation, and since the first was believed to be less toxic for the heart than the latter (Santesson(18), Valey and Waller(19)), it was substituted in therapeutics for the latter According to Lewis and his pupils(20) Quinidine lowers the S A rate, reduces the rate of conduction in auricle and ventricle, depresses the A V conduction and lengthens the absolute refractory period of the auricular muscle In view of these effects, it seemed desirable to test this alkaloid in the heart-lung preparation as well A dose of 0 0066-0 01 gm Quinidine sulphate was found to produce in addition to a bradycardia, a very well-marked heart dilatation (Fig 12) The slowing of the heart beat would cause in itself

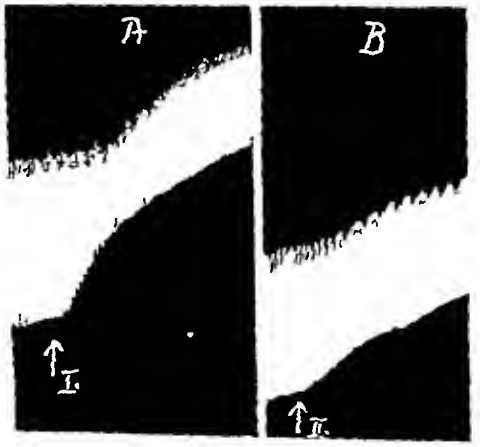

Fig 12 Quinidine.

A = Heart normal Temp 37 0° C Heart rate = 168 per min. Outflow = 480 c c. per min. $a.P$ = 110 mm. Hg $v.P$ = 7 0 cm. H_2O Heart's weight = 83 gm. At ↑I 0·0066 gm. Quinidine sulphate was added. Heart dilating At ↑II 0 0066 gm. Quinidine sulphate was added.

a dilatation by prolonging the diastolic filling of the heart In order to investigate how far this is responsible for the heart dilatation the heart was artificially driven by means of rhythmic electrical stimuli during the whole of an experiment, before, during and after the administration of Quinidine, at a rate corresponding with the original rate of the heart

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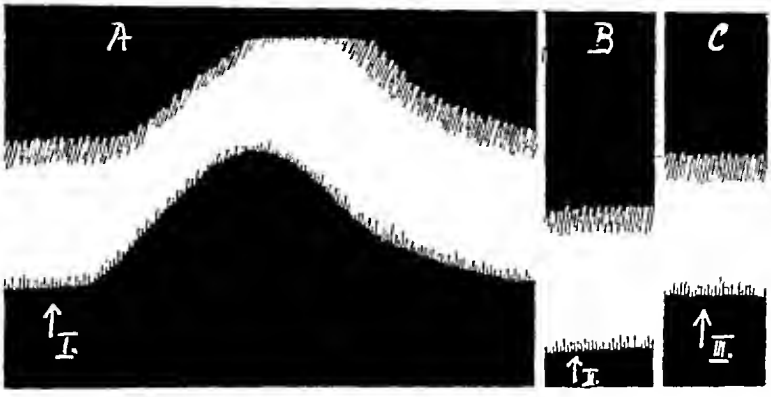

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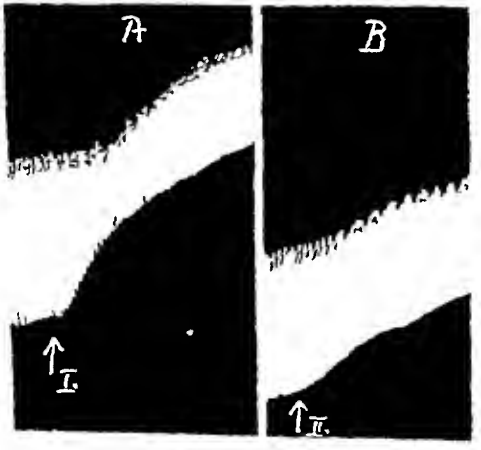

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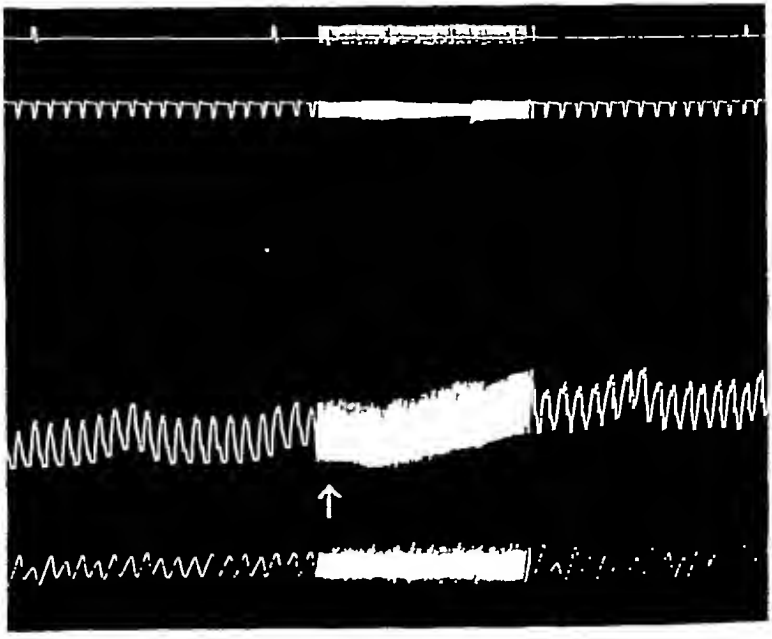

Fig 13 Quinidine.

Temp 37 0° C Heart artificially driven at a rate of 180 per min. Outflow = 429 c.c. per min. B.P = 115 mm Hg V P = 6 0 cm. H_2O Heart s weight = 78 gm. At ↑ 0 01 gm. Quinidine sulphate was added.

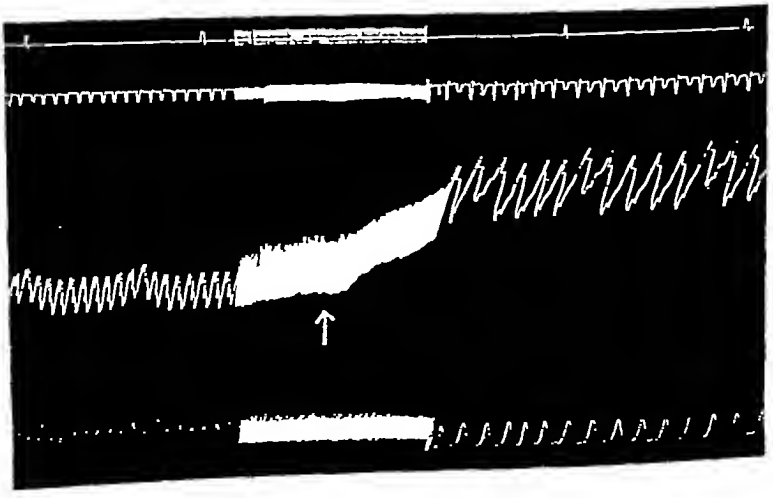

Fig 14. Quinidine

Same exp as Fig 13 but later At † 0-0166 gm Quinidine sulphate was added
The ventricles do not follow the artificially driven auricles.

As can be seen in Figs 13 and 14, though the complicating factor of bradycardia is excluded, the heart dilates after Quinidine In Fig 14 it can be observed, that after a second dose of Quinidine the ventricles are unable to follow the artificially driven auricles Quinidine had no effect on the coronary circulation (Table X), unless given in a large dose, which caused after a preliminary dilatation, constriction of the coronary vessels

TABLE X.

Temp 37 0° C Heart rate=144 per min. B.P =110 mm. Hg
Heart s weight=77 0 gm.

Time	System output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary flow (calc.) c.c per min	Addition of
4 40	—	29	48	
4.45	390	29	48	
5 52	—	30	50	
5 53	—	—	—	0 0066 gm. Quinidine sulph.
5 56	—	31	52	
5 59	390	31	52	
6 03	—	31	52	
6 05	—	31	52	
6 06	—	—	—	0·01 gm. Quinidine sulph.
6 07	—	36	60	Heart dilatation. Bradycardia
6 10	350	25	42	
6 13	—	24	40	
6 15	—	21	35	

DISCUSSION

Reviewing the experiments, it can be said, that among the heart-tonics Digitalis and Caffeine really produce a tonic effect because they enable the heart to expel the same amount of blood as before, with a smaller heart volume, the rhythm, venous inflow and arterial resistance being unchanged The difference in their effect is only that after Digitalis the tonic effect develops slowly but lasts long, after Caffeine it is immediately apparent but has only a transitory character Camphor and Strychnine, on the other hand, have no such effect, in the case of Camphor indeed, the effect is just the reverse, since it dilates the heart

Among the other substances investigated, insulin produced a well-marked and lasting tonic effect, but this effect could be obtained only once, the second and further doses being ineffective Pituitrin, on the other hand, dilated the heart its effect appeared immediately after its administration, was of a transitory character, and usually could not be repeated. Quinidine, apart from bradycardia, produced also a heart dilatation, which was observed even after the exclusion of the change in

the heart rate Sodium nitrite dilated the heart and Amyl nitrite had no effect at all on the heart muscle fibres

As regards the mechanism of the tonic action, it may be considered as the direct opposite of that which occurs during the dilatation of the heart As was shown by Starling and his pupils, if the work presented to the heart is suddenly increased, the heart fails for a time, and therefore gradually dilates Immediately after the increase of the arterial resistance, the ventricle does not empty itself as completely as before, so that at the end of the systole the heart still contains blood, or, more blood than at the end of a previous beat Since the ventricle is receiving a constant inflow from the venous reservoir, it is more distended in the next diastole than before In the next systole the output is increased, but still not equal to the inflow, and this disparity becomes smaller at each beat for 3–10 beats, the output steadily increasing and the heart steadily dilating, until a new balance is attained, the output becoming again equal to the inflow, but the heart volume being increased If the venous inflow is increased, the changes produced are somewhat similar the output steadily increasing from its normal amount and the heart steadily dilating until it reaches again a condition in which the output becomes equal to the inflow with increased heart volume

In our experiments, whether the work of the heart was augmented by increasing the arterial resistance or the venous inflow or both, when the tonic effect begins to be apparent, there is a transitory increase in the output and a gradual decrease in the heart volume until it arrives at a state in which the output is again equal to the inflow, but the heart volume is smaller Starling and Visscher have shown that as the heart tires and dilates, in order to maintain the same work as before the total energy liberated increases with the increase in volume, and the mechanical efficiency of the heart accordingly diminishes This rule was established by observations on hearts not treated with drugs, and it is a question whether it could be applied also to hearts under such influence In the present paper this has not been investigated, oxygen consumption not being determined, so that the question cannot be answered definitely It is hoped that these determinations may be made on a later occasion If this rule is found to be valid even after the administration of a tonic, which is quite conceivable, this would mean that, as the heart volume becomes smaller, its functional capacity improves, its mechanical efficiency being increased

In any case the decrease in volume obtained is the important direct therapeutic effect of the tonics upon the heart The therapeutic effect on

the conductivity, which is according to Cushny (21) and Lewis (22) an effect partly on muscle and partly on the vagus, will not be discussed here The conception which can still be found in various textbooks and papers, that one of the most important direct effects of Digitalis upon the heart is to increase the diastolic volume, is certainly incorrect Just as a skeletal muscle has no power to lengthen itself actively after the contraction, but has to be lengthened by the application of some extending force, so, in the same way, the ventricles cannot dilate of themselves after systole, but must be distended by the inflow of blood, and the degree of this distension depends on the rate and pressure at which the blood enters the heart, the diastole of the ventricles being terminated by the next systole In the heart-lung preparation the rate and pressure of the inflow and the rate of the beat being all constant no increase in diastole could be observed after Digitalis, but in experiments performed in other ways and especially in experiments on the whole animal, this effect may be observed, because the slowing of the rhythm produced by Digitalis indirectly through the nervous system prolongs the time for the diastolic filling Even after exclusion of the vagus action by atropine this increase of diastolic volume may be observed, because the general constriction of the vessels by Digitalis diminishes the total volume of the vascular system —thereby raises the pressure of the inflow But these are secondary effects, and not the direct effect on the heart itself

Further, from the results obtained, the conclusion must be drawn that with the exception of Pituitrin there exists no definite connection between the tonic effect of a drug on the heart muscle and its effect on the coronary vessels, since some had the same effects on the heart muscle but produced a quite different effect on the coronary flow, while others having the same effect on the coronary circulation produced a different effect on the heart muscle Digitalis and Caffeine, for example, are heart-tonics and dilate the coronary vessels, but Insulin, which had the same effect on the heart muscle had either no effect on the coronary flow or diminished it if given in larger doses (10 units), which still produced a tonic effect On the other hand camphor dilated the heart and increased the coronary flow Pituitrin and Quinidine also produced a heart dilatation, but constricted the coronary vessels (in the case of the latter this was observed only after larger doses, small doses having no effect on the coronary flow) Even the two nitrites, though they had almost the same effect on the coronary circulation, behaved differently towards the heart muscle, Sodium nitrite dilating the heart while Amyl nitrite had no effect at all In the case of Pituitrin the appearance and duration of the two effects

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made it very probable that they are in connection, the constriction of the coronary vessels being a probable cause of the heart dilatation.

Concerning the effect on the coronary flow it has to be remembered that in the denervated heart-lung preparation the coronary vessels are deprived of the nervous control, which might cause a great difference in the effects of these drugs It would be of interest to investigate the effects of these drugs upon the coronary vessels in the innervated heart-lung preparation also

SUMMARY

1 Provided the heart rate, the venous inflow and the arterial resistance are maintained constant, the tonic action of drugs upon the heart is revealed in the heart-lung preparation by a diminution in the diastolic and systolic volume of the ventricles

2 Judged by this criterion Digitalis, Caffeine and Insulin produce a tonic effect, enabling the heart to expel the same amount of blood with a smaller average heart volume This effect is, after Digitalis, delayed but lasting, after Caffeine relatively rapid in onset and evanescent, and after Insulin it could be observed only once in each preparation

3 Camphor, Sodium nitrite, Pituitrin and Quinidine produce dilatation of the heart

4 Strychnine and Amyl nitrite have no effect on the tone of the heart

5 Digitalis, Caffeine, Camphor and the Nitrites increase the coronary flow, Nitrites and Caffeine in a very high degree, Digitalis and Camphor only slightly

6 Pituitrin, Quinidine and Insulin diminish the coronary flow, the two latter only in larger doses, in smaller doses these are without effect

7 With the exception of Pituitrin there exists no obvious relationship between the tonic effect and that produced on the coronary flow

I take this opportunity of recording my lasting debt of gratitude to the late Prof Starling for help and inspiration in this work and in a wider field

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THE CAUSATION OF THE ANŒSTROUS PERIOD

By A S PARKES (*Beit Memorial Research Fellow*)
and F W R BRAMBELL (*Lecturer in Zoology, King's College, London*)

(*From the Department of Physiology and Biochemistry, and the Department of Anatomy, University College, London*)

I Introduction

Under natural conditions the majority of mammals have definite anœstrous periods, during which the reproductive processes are in abeyance Further it is known that under laboratory conditions of constant temperature and food supply, and also to a lesser extent under conditions of domestication, mammals will breed throughout a much greater seasonal range In most laboratory rodents, for instance, reproduction will take place throughout the entire year, although a winter anœstrus is characteristic of the wild types It would seem that the constant temperature under laboratory conditions might be a factor in the disappearance of anœstrus (1), and this suggests the obvious test of transferring the animals to winter atmospheric temperatures Allen (2) reports negative results of experiments upon a small number of mice While our experiments were in progress Lee (3) reported an investigation of a somewhat similar nature on rats, the animals being left in a natural atmospheric temperature varying from − 6° C to − 3° C At − 6° C the length of cycle was much increased, but at − 3° C no very marked increase (1 7 days) was found

In the present series of experiments we first examined, during not less than four weeks, the œstrous cycles of 30 selected mice kept in a constant artificial temperature of 16 5° C to 18 5° C during January–February, the animals were then put for 4 weeks into an outside shed, no attempt being made to regulate the temperature, which ranged between a daily maximum and minimum of 5° C to 10° C As this produced no regular effect on the periodicity of œstrus, the animals were then put into cold store where the temperature ranged from − 1° C to 1° C After 45 days certain of the animals were mated, and were removed from cold store if and when pregnancy ensued

The detection of œstrus, copulation, etc was by the methods previously described (4) During the whole of the experiments an unlimited food supply was allowed

II Experimental Results

Control experiments One hundred and eighty-two œstrous cycles were observed before treatment The length of the dioestrous intervals in these cycles is shown in the following frequency distribution

Table I Length of dioestrous intervals at laboratory temperatures (16 5 to 18 5° C.) before treatment.

Length of interval in days	No observed	Total days occupied
2	52	104
3	53	159
4	31	124
5	17	85
6	11	66
7	2	14
8	2	16
9	5	45
10	3	30
12	4	48
16	1	16
25	1	25
Total	182	732

The mean length of dioestrus before treatment was thus 4 02 days, and the distribution gives $\sigma = 2\ 81$ and a p e of $\pm 0\ 14$

Length of dioestrus at outdoor temperatures Under the outside shed conditions 86 dioestrous intervals were observed[1] The average length of these intervals was $5\ 65 \pm 0\ 30$ This is longer by $1\ 63 \pm 0\ 33$ days than that found for the pre-treatment cycles, but as shown by Table II, the most obvious result of putting the animals under outdoor conditions was to increase the variability of the length of dioestrus

The variability in this series ($\sigma = 4\ 16$) is definitely greater than that found in the pre treatment cycles ($\sigma = 2\ 81$)

Length of dioestrus in artificially low temperatures Whilst the animals were kept in cold store, 201 dioestrous intervals were observed The mean length was $4\ 97 \pm 0\ 171$ days, which is only 0 95 days longer than before treatment and which suggests that no effect whatever had been produced by the low environmental temperature Examination of the individual

[1] As an arbitrary method of assessment the dioestrous interval was placed to one period or the other according to the incidence of its mid point

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sence of such [illegible]

is, however, much higher at the lower [illegible]
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uch liberal food supply can be available
s been shown experimentally [illegible] that [illegible]
s even at normal temperatures to increase
would appear quite possible that the [illegible]

No. of oestrous periods without copulation	Size of litter	Sex of young: Males	Sex of young: Females
0	7	4	3
0	Definitely pregnant at 13 days but apparently re-absorbed		
0	6 in uterus when killed	–	–
1	8	3	5
0	6	4	2
1	3 (some eaten)	3	–
0	5	4	1
0	Pregnant when killed	–	–
4	–	–	–
2	4	2	2
1	9	4	5
1	–	–	–
0	8	5	3
0	Aborted	–	–
0	–	–	–
0	–	–	–
0	2	2	0
0	Definitely pregnant at 12 days but re-absorbed		
1	Definitely pregnant at 11 days but re-absorbed		
0	Died in parturition	–	–
1	–		–
0	–	–	–
0	7	3	4
0	5	2	3
0	Definitely pregnant at 15 days but re-absorbed		

TABLE II Frequency distribution of length of dioestrus under outside conditions (5° C. to 10° C.)

Length of dioestrus in days	No. of intervals	Total days
2	10	20
3	21	63
4	13	52
5	19	95
6	2	12
7	4	28
8	2	16
9	2	18
10	3	30
11	2	22
12	2	24
13	1	13
14	1	14
18	1	18
19	1	19
20	1	20
22	1	22
Total	86	486

oestrous histories, however, showed that in nearly all cases the first dioestrus in cold store was much prolonged. The dioestrous intervals occurring in cold store were therefore grouped according to the incidence of their mid-point. The results were as follows:

TABLE III Length of cycle according to time in cold store

Weeks in cold store	Length of dioestrus 2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total intervals	Mean length
1	1	1	1	1	–	–	1	4	2	3	–	3	–	2	4	1	1	25	11·16 ± 0·59
2	2	4	7	3	1	–	–	–	–	–	–	–	–	–	–	–	–	17	
3	3	3	2	7	2	–	3	1	2	1	3	–	–	–	–	–	–	27	
4	14	8	5	–	1	1	–	1	–	–	–	–	–	–	–	–	–	30	
5	3	6	13	2	1	–	–	–	2	–	–	1	–	–	–	–	–	28	4·09 ± 0·12
6	3	14	9	1	–	1	1	–	–	–	–	–	–	1	–	–	–	30	
7	4	13	5	–	1	–	–	–	–	1	–	–	–	–	–	–	–	24	
8	3	11	5	1	–	–	–	—	–	–	–		–	–	–	–	–	20	
Total	33	60	47	15	6	2	5	6	6	5	3	4	0	3	4	1	1	201	4·97 ± 0·171

The initial increase in length of dioestrus is obvious from this table, and comparison of the initial and subsequent length of dioestrus in the cold store with the pre-treatment length gives the following results:

Length of dioestrus during	Difference from pre-treatment length	P.e. of difference	Difference/p.e.
1st week in cold store	+7·14 days	±0·61	11·7
2nd to 8th weeks in cold store	+0·07 days	±0·18	0·389

The following conclusions may be drawn from these results

(*a*) The first effect of much reduced temperature is to lengthen the dioestrous interval.

(*b*) Subsequently, however, the length of cycle becomes indistinguishable from the normal, and no sign of anœstrus is found.

There is, therefore, no simulation of the prolonged winter anœstrus of wild rodents, the subsequent return of the cycle to normal suggests that the experimental animals were able to adjust themselves to the low temperature, and that in the absence of such adjustment anœstrus might have resulted

The food consumption was, however, much higher at the lower temperature, and it was perhaps this which made the adjustment possible With the wild mammal no such liberal food supply can be available during the winter, and it has been shown experimentally (5, 6) that an insufficient food supply leads even at normal temperatures to increase in the length of dioestrus It would appear quite possible that the anœs-

No of animal	No of infertile copulations	No of œstrous periods without copulation	Size of litter	Sex of young: Males	Sex of young: Females
CC 3	0	0	7	4	3
CC 5	0	0	Definitely pregnant at 13 days but apparently re absorbed		
CC 9	0	0	6 in uterus when killed	–	–
CC 10	0	1	8	3	5
CC 14	0	0	6	4	2
CC 15	0	1	3 (some eaten)	3	–
CC 16	0	0	5	4	1
CC 18	0	0	Pregnant when killed	–	–
CC 19	0	4	–	–	–
CC 22	0	2	4	2	2
CC 23	0	1	9	4	5
CC 25	1	1	–	–	–
CC 27	0	0	8	5	3
CC 28	0	0	Aborted	–	–
CC 29	2	0	–	–	–
CC 32	2	0	–	–	–
CC 34	0	0	2	2	0
CC 38	0	0	Definitely pregnant at 12 days but re absorbed		
CC 39	0	1	Definitely pregnant at 11 days but re absorbed		
CC 40	0	0	Died in parturition	–	–
CC 42	1	1	–	–	–
CC 44	3	0	–	–	–
CC 45	0	0	7	3	4
CC 46	0	0	5	2	3
CC 47	0	0	Definitely pregnant at 15 days but re absorbed		

TABLE II Frequency distribution of length of dioestrus under outside conditions (5° C to 10° C)

Length of dioestrus in days	No of intervals	Total days
2	10	20
3	21	63
4	13	52
5	19	95
6	2	12
7	4	28
8	2	16
9	2	18
10	3	30
11	2	22
12	2	24
13	1	13
14	1	14
18	1	18
19	1	19
20	1	20
22	1	22
Total	86	486

oestrous histories, however, showed that in nearly all cases the first dioestrus in cold store was much prolonged The dioestrous intervals occurring in cold store were therefore grouped according to the incidence of their mid-point The results were as follows

TABLE III Length of cycle according to time in cold store

Weeks in cold store	Length of dioestrus 2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total intervals	Mean length
1	1	1	1	1	–	–	1	4	2	3	–	3	–	2	4	1	1	25	11 16 ± 0 59
2	2	4	7	3	1	–	–	–	–	–	–	–	–	–	–	–	–	17	
3	3	3	2	7	2	–	3	1	2	1	3	–	–	–	–	–	–	27	
4	14	8	5	–	1	1	–	1	–	–	–	–	–	–	–	–	–	30	
5	3	6	13	2	1	–	–	–	2	–	–	1	–	–	–	–	–	28	4 09 ± 0 12
6	3	14	9	1	–	1	1	–	–	–	–	–	–	1	–	–	–	30	
7	4	13	5	–	1	–	–	–	1	–	–	–	–	–	–	–	–	24	
8	3	11	5	1	–	–	–	—	–	–	–			–	–	–	–	20	
Total	33	60	47	15	6	2	5	6	6	5	3	4	0	3	4	1	1	201	4 97 ± 0 171

The initial increase in length of dioestrus is obvious from this table, and comparison of the initial and subsequent length of dioestrus in the cold store with the pre-treatment length gives the following results

Length of dioestrus during	Difference from pre treatment length	P e of difference	Difference/p e
1st week in cold store	+7 14 days	±0 61	11 7
2nd to 8th weeks in cold store	+0 07 days	±0 18	0·389

THE EFFECT OF TEMPERATURE ON THE ACIDITY OF THE SKIN

H. C. BAZETT AND B. McGLONE

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THE studies of Stadie and Martin(1), Austin and Cullen(2) and Stadie, Austin and Robinson(3) have demonstrated that blood *in vitro* becomes more acid when it is warmed, unless CO_2 can escape. It is consequently important to decide whether the same changes occur *in vivo*, and if so whether the vascular dilatation produced by heat is in any way dependent on such changes. Rous and his co-workers(4) have described the injection of large amounts of indicator dyes into mice and other animals in order to determine approximately the acidity of the tissues. It has been found that his method may be adapted to cats and used for the estimation of changes in acidity in the skin.

Method. White cats of 3 to 3½ kilos weight have been used. They were decerebrated under a brief chloroform-ether anæsthesia by the Sherrington guillotine method(5), care being taken to divide the brain stem between the posterior colliculi and the anterior margin of the pons, so as to have a quiescent preparation. The two fore limbs were then shaved between the elbow and wrist joints, and the hyperæmic response to various conditions was estimated by comparing the colour of the skin of the two limbs. After these observations had been made about 30 c.c. of a 2 p.c. solution (approximately neutral) of phenolsulphonephthalein was injected intravenously into a vein of the hind limb. After an injection the cats had a purplish pink colour, which was readily visible through the hair and which was intense on the shaved areas. The colour developed within three-quarters of a minute and reached its maximum in less than two minutes. The dye was rapidly excreted by the kidneys and the colour was fading rapidly two to four hours later. The colour of the skin, as a whole, was definitely towards the alkaline side of the indicator range, but that of the ears, occasionally was quite yellow, since both common carotid arteries had been tied. Afterwards the ears usually resumed a more alkaline colour. The shaved legs, which were cold as a result of exposure and evaporation of water, also had a

trous period of the wild rodent results from decrease in both temperature and food supply

Breeding performance at − 1 to + 1° C The above table sums up the performance of the 25 animals which were mated in the cold store with normal males

In these mated animals 12 œstrous periods unattended by copulation were observed, but only one animal failed to copulate at any time Infertile copulations (nine in all) were observed in five mice Nineteen of the 25 thus became pregnant The excessive number of cases (five) where the pregnancy terminated prematurely by re-absorption or abortion of all the fœtuses was probably due to the sudden change of environment on being taken from the cold store

III Conclusions and Summary

(1) A small decrease in environmental temperature has no marked effect on the œstrous cycle in mice except that the variability in length is somewhat increased

(2) A considerable decrease, however, from the ordinary temperature of 18° C to a mean of about 0° C causes an initial increase in the length of diœstrus (4 02 ± 0 14 to 11 16 ± 0 59) Very shortly, however, the normal periodicity of œstrus is restored, and even prolonged exposure to the low temperature produces no further effects on the reproductive processes When mated the animals are normally fertile

(3) The prolonged winter anœstrus of wild rodents is probably to be attributed to a diminished food supply under conditions of temperature which, for the maintenance of normal function, would require an increase in food

The animals used for the investigation recorded above were drawn from the colony maintained with the aid of a grant to one of us (A S P) from the Medical Research Council, to whom our thanks are due

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least 25 min before it was used for any further experimentation. If stasis had been applied for 20 min. these periods were extended to 15 and 30 min. In addition, the colour of the control leg was determined not to be altering appreciably before any further experiments were made

Two abbreviated protocols are appended and will serve to illustrate the character of the experiments, the time intervals allowed and the type of change noted.

Protocol 1 Cat male, 3·2 kg 9·35 to 9·50 decerebrated. 10·19 rectal temperature 39·2 Slight hæmorrhage from brain stem subsiding 10·25 opisthotonos which disappeared later 10·30 shaved and "Piva Rocci" applied. 10·59 with both forelegs in air an arrest of circulation was maintained by 150–180 mm. Hg for 5 min. Thirty seconds after release the shaved areas and the paws became hyperæmic at 1 min. 30 sec. the hyperæmia persisted without perceptible change and at 2 min. 45 sec. a trace of hyperæmia was present

Table I details a series of consecutive arrests of circulation for varying periods, with the forelegs immersed in water at an intermediate temperature Time in minutes ( ) and seconds (″)

12.50 rectal temperature 39·0 1.38 30 c.c. 2 p.c. solution of phenol red injected. The colour of the skin matched that of a standard tube of *p*H 7·6 1·43 rectal temperature 39·7

Table II details a series of consecutive arrests of circulation for varying periods, with the forelegs fully immersed in water

Protocol 2. Cat female 3·1 kg 10·15 to 10·39 decerebrated. 11·17 rectal temperature 38·0 11·20 shaved. Riva Rocci apparatus applied to both forelegs above the elbow but on the right the bandage covered part of the elbow Both legs at this time showed slight congestion.

Table III details a series of consecutive circulatory arrests of the same duration but with the temperature of the bath varied.

1·12 rectal temperature 38·0 2·21 rectal temperature 37·0° 2.45 30 c.c. 2 p.c. solution of phenol red injected. 2·49 both forelegs into bath of 39·5

Table IV details a second series of consecutive arrests circulatory for comparison with the previous series.

4·46 both forelegs were partially immersed in water of 40·5 The acidity of the skin of immersed portion definitely greater than that of the skin which was not in the bath. 4·49 the forelegs were completely immersed, the right in a bath at 36 the left in one at 18·5 The line of previous submergence was recognisable on both forelegs by a greater intensity of colour which within 1 min. had disappeared on the right foreleg (36) though persistent on the left (18·5) This increased colour in the left leg disappeared after 6 min. to 7 min. 5·12 a $\frac{1}{10}$ solution of ergamine acid phosphate (histamine) was introduced into the skin and subcutaneous region of the left leg by the method of Lewis and Grant(5) The lower part of the right leg was scalded with boiling water No change of colour was noted in either limb

5·20 application to left foreleg of histamine repeated, employing $\frac{1}{10}$ solution, and again the same area of the right foreleg was scalded. Circulation arrested under pressure of 200 mm. Hg 5·24 arrest terminated the right foreleg definitely acid, most marked where previously scalded. 5·25 the centres of the histamine affected areas appeared to have a more yellow tint than the rest of the leg 5·27 circulation arrested in bath 31·5° 5·31 arrest terminated. Contrast between inflamed and normal areas unchanged during and after arrest

definitely purplish tint (similar to that of a standard solution of the dye of a concentration of 0 001 p c with a *p*H of 7 6 at 20° C and contained in a test tube of 13 3 mm internal diameter) Where the skin had been abraded in the process of shaving, the colour was of a deeper purple tint, either through removal of cuticle, which would otherwise dull the colour, or, more probably, through an actual increase of alkalinity due to loss of CO_2 from the surface The wounds due to the operative procedures left uncovered by skin assumed a very alkaline purplish colour, suggesting a *p*H of at least 7 8 or more The pads of the feet, where unpigmented, had a less purplish tint than the skin of the shaved areas

Measurement of subdermal temperatures in the wet shaved forelegs in one animal made by loop-thermocouples (6) indicated temperatures of 23° to 27° C The rectal temperature of the cats has been maintained as constant as possible and has usually remained between the limits of 36° and 39°

For purposes of determining the effect of temperature, both before and after injection, the cats were suspended with their heads raised and the two fore limbs immersed in water in rectangular glass museum jars, the water was not stirred except immediately after the initial insertion Behind the glass jar an opal glass plate was adjusted, so as to afford a constant background One of the limbs was used as a control, and colour differences between the two limbs were noted, an attempt was made to express any difference in tint according to a scale of colour variation with *p*H at 20° C in standard tubes of the type already mentioned Such colours are mentioned so as to give an approximate idea of the actual tint, it was not possible to estimate the exact *p*H level, since a colour match was prevented by the opaqueness of the tissues, by irregular errors introduced by varying amounts and degrees of saturation of hæmoglobin, as well as by unmeasurable salt and protein errors The colour changes were, however, often definite, and must have indicated the direction of the changes in acidity and approximately their magnitude, in spite of the above-mentioned errors

The effect of circulatory arrest on the acidity of the skin has been tested "Riva Rocci bags" made of rubber finger stalls were tied on to glass tubes and bandaged lightly over the humerus and elbow joint with a 1-inch bandage This system was connected to a mercury manometer The pressure could be raised to over 200 mm in less than two seconds Stasis was maintained for varying periods up to 20 min. The legs were employed alternately If stasis had been applied for 5 min , an interval of at least 12 min was left before that leg was used as a control, and at

TABLE III

Time arrest initiated Hr	Mn	Duration of arrest	Pressure within compressing system Mm Hg	Leg used in experiment	Temp of bath °C	Changes during period of stasis	Period of release: Appearance of flush on release	Maximum flush	Fading begins	Fading advanced	Colour normal
11	35	10	200	Left	40	Marked cyanosis	20″	40″	50″	18′	—
12	10	10	200	Right	41	Very marked cyanosis	30″	120″	2′ 30″	9	11′ 30″
12	51	10	200	Left	30	Cyanosis developed slowly 30–60″ Definite cyanosis 1′ 15″	15″	60″	1′ 15″	8	10′ 30″
1	10	10′	200	Right	30	Cyanosis 15″ Marked cyanosis 60–90″	15″	75″	2′ 30″	5′	5′ 30″?

TABLE IV

Time arrest initiated Hr	Mn	Duration of arrest	Pressure within compressing system Mm Hg	Leg used in experiment	Temp of bath °C	Changes during period of stasis: Acidity detectable	Changes during period of stasis: Ultimate colour obtained Maximum	Period of Release: Increase in acid colour	Reversal towards alkalinity	Trace of acidity	Return to normal	Remarks
2	51	10′	200	Left	30 5	90″	*p*H 7 5–7 4	60″	90–105″	4′ 45″–5′	7′ 30″	Control (right) leg *p*H 7 6
3	15	10′	200	Right	29 5	—	*p*H 7 4	45″	105″	7–8′	8′–8′ 30″	Control (left) leg a little on the acid side of *p*H 7 6
3	51	10′	200	Left	40 5	60″	*p*H 7 0	30″	45–60″	10′	10′ 30″?	Control (right) *p*H 7 4
4	18	10′	200	Right	41 0	45–60″	*p*H 7 0	15″	15–60″	6′	5′ 30″–6′ 30″	Control (left) *p*H 7 4

TABLE I.

Time arrest initiated		Duration of arrest	Pressure within compressing system Mm Hg	Leg used in experiment	Temp of bath °C	Changes during period of stasis	Period of release				
Hr	Min						Appearance of flush on release	Maximum flush	Fading begins	Fading advanced	Colour normal
11	20	1′	160	Left	34 5	—	10–11″	45″	45–75″	—	75–80″
11	36	5′	200	Right	36 0	Skin first pale then cyanotic	10–12″	37–39″	60″	—	90″?
11	58	10′	180	Left	35 0	Cyanosis perceptible on shaved skin 30″ and on dorsal area of toes 4′ 30″ after arrest	0–10″	20–30″	70–120″	4′ 45″–5′ 30″	6′ 30″
12	25	10	200	Right	35 0	Cyanosis perceptible 73″	0–10″	30–80″	70–120″	4′ 30″–6′ 30″	8′ 30″

TABLE II.

Time arrest initiated		Duration of arrest	Pressure within compressing system Mm Hg	Leg used in experiment	Temp of bath °C	Changes during period of stasis		Period of release				Remarks
Hr	Min					Acidity detectable	Ultimate colour obtained Maximum	Increase in acid colour	Reversal towards alkalinity	Trace of acidity	Return to normal	
1	54	30″	200	Left	34 5	—	—	30″ ?	—	—	—	After this test right leg approximate *p*H 7 6, left *p*H 7 5
1	57	1	200	Right	34 5	—	—	10–20″	—	60–95″	—	
2	05	5	200	Left	34 5	16–20″	*p*H 7 0	6–25″	60–80″	4′ 30″	5	
2	25	10	200	Right	35 5	20–30″	*p*H 6 9	5″	60″	4′ 30″	5	
2	53	15	200	Left	36 0	15–45″	*p*H 6 8	immediate	11–45″	6′ 30″	11–12	
3	35	The right leg was placed in a bath of 12 5° with the circulation intact, when equilibrium was established the colour present corresponded to a standard of *p*H 7 5 Similarly the left leg in a bath of 40 5° had a colour on this basis of *p*H 7 2 Arrest of circulation gave the following results										
3	52	10	200	Right	12 5	Possibly slight change to acid side		Any further change not perceptible				
3	52	10	200	Left	40 5	30″	Lower than *p*H 6 8	—	18–20″	2′ 30″	3′ 30″–4′ 30″	

TABLE III

Time arrest initiated Hr	Min	Duration of arrest	Pressure within compressing system Mm Hg	Leg used in experiment	Temp of bath °C	Changes during period of stasis	Period of release: Appearance of flush on release	Maximum flush	Fading begins	Fading advanced	Colour normal
11	35	10	200	Left	40	Marked cyanosis	20″	30″	50″	18′	—
12	10	10′	200	Right	41	Very marked cyanosis	30″	120″	2′ 30	9	11′ 30″
12	51	10	200	Left	30	Cyanosis developed slowly 30–60″ Definite cyanosis 1′ 45″	45″	60″	1′ 45″	8	10′ 30″
1	16	10′	200	Right	30	Cyanosis 45″ Marked cyanosis 60–90″	45″	75″	2′ 30″	5	5′ 30″?

TABLE IV

Time arrest initiated Hr	Min	Duration of arrest	Pressure within compressing system Mm Hg	Leg used in experiment	Temp of bath °C	Changes during period of stasis: Acidity detectable	Ultimate colour obtained Maximum	Period of Release: Increase in acid colour	Reversal towards alkalinity	Trace of acidity	Return to normal	Remarks
2	51	10′	200	Left	30 5	90″	*p*H 7 5–7 4	60″	90–105″	4′ 45″–5′	7′ 30″	Control (right) leg *p*H 7 6
3	15	10′	200	Right	29 5	—	*p*H 7 4	45″	105″	7–8	8′–8′ 30″	Control (left) leg a little on the acid side of *p*H 7 6
3	51	10′	200	Left	40 5	60″	*p*H 7 0	30″	45–60″	10	10′ 30″?	Control (right) *p*H 7 4
4	18	10′	200	Right	41 0	45–60″	*p*H 7 0	45″	45–60″	6	5 30″–6 30″	Control (left) *p*H 7 4

TABLE I.

Time arrest initiated		Duration of arrest	Pressure within com pressing system Mm Hg	Leg used in ex periment	Temp of bath ° C	Changes during period of stasis	Period of release				
Hr	Min						Appearance of flush on release	Maximum flush	Fading begins	Fading advanced	Colour normal
11	20	1′	160	Left	34 5	—	10–11″	45″	45–75″	—	75–80″
11	36	5′	200	Right	36 0	Skin first pale then cyanotic	10–12″	37–30″	60″	—	90″?
11	58	10′	180	Left	35 0	Cyanosis perceptible on shaved skin 30″ and on dorsal area of toes 4′ 30″ after arrest	0–10″	20–30″	70–120″	4′ 45″–5′ 30″	6′ 30″
12	25	10	200	Right	35 0	Cyanosis perceptible 73″	9–10″	30–80″	70–120″	4′ 30″–6′ 30″	8′ 30″

TABLE II

Time arrest initiated		Duration of arrest	Pressure within com pressing system Mm Hg	Leg used in ex periment	Temp of bath ° C	Changes during period of stasis		Period of release				Remarks
Hr	Min					Acidity detectable	Ultimate colour obtained Maximum	Increase in acid colour	Reversal towards alkalinity	Trace of acidity	Return to normal	
1	54	30″	200	Left	34 5	—	—	30″ ?	—	—	—	After this test right leg approximate pH 7 6 left pH 7 5
1	57	1	200	Right	34 5	—	—	10–20″	—	—	—	
2	05	5	200	Left	34 5	10–20″	pH 7 0	6–25″	60–80″	60–95″	—	
2	25	10	200	Right	35 5	20–30″	pH 6 9	5″	60″	4′ 30″	5′	
2	53	15	200	Left	36 0	15–45″	pH 6 8	immediate	11–45″	6′ 30″	11–12	
3	35	The right leg was placed in a bath of 12 5° with the circulation intact when equilibrium was established the colour present corresponded to a standard of pH 7 5 Similarly the left leg in a bath of 40 5° had a colour on this basis of pH 7 2 Arrest of circulation gave the following results										
3	52	10	200	Right	12 5	Possibly slight change to acid side		Any further change not perceptible				
3	52	10	200	Left	40 5	30″	Lower than pH 6 8	—	18–20″	2′ 30″	3′ 30″–4′ 30″	

photographically No difference in colour could be detected unless the temperature difference between the two legs exceeded 12° to 15°

It may therefore be concluded that in spite of the well recognised changes in the circulation rate, on exposure to cold the acidity of the skin is reduced while on warming it is increased

Acidity developed during arrest of the circulation at intermediate temperatures If the legs were immersed side by side in water at a temperature of 31° to 35° (usually 32° to 33°) and the circulation was arrested in one limb the colour gradually altered towards a yellowish tint in spite of the simultaneous development of cyanosis Protocol 1 gives an example of this in an experiment where the acidity developed was somewhat greater than that usually observed The acidity reached during stasis, and the time required on the release of the circulation to return to the initial colour varied with the duration of stasis the time values recorded were not very different from those noted for reactive hyperæmia following similar occlusions before the injection of the dye Though no exact parallelism in duration was to be observed, both phenomena could often be distinguished for 5 to 10 min The absence of any exact parallelism is not significant, since the condition of the animal was not necessarily the same in the two series of experiments, and in both cases the colour differences were difficult to time exactly The reactive hyperæmia was less conspicuous in the cats than in experiments on man. Both the development and disappearance of the acid colour in the skin showed a somewhat patchy distribution the skin was everywhere acid, but the degree of acidity apparently differed in different areas

The effect of temperature on the acidity developed during circulatory arrest If one leg was immersed in cold water and the other in warm and if after some minutes the circulation was arrested in both simultaneously the warmed leg rapidly developed an increased acidity while the cooled leg showed no appreciable change in colour (see Protocol 1) In one animal a series of circulatory arrests were made at two temperatures both before and after injection of the dye, and the data are presented in Protocol 2 An intensification of the reactive hyperæmia by warmth was noted (confirming the earlier observations of Lewis and Grant(8) on man), and at the higher temperature on this occasion it also lasted longer after release The acidity developed was much greater at the higher temperature, and its duration on release was also greater, though the time differences were less marked than in the series with reactive hyperæmia It may be noted that the acidity developed in all the experiments was greater the higher the temperature of the bath, but that sometimes the period of recovery

Results

Acidity in the skin as affected by temperature with the circulation intact Cooling of one limb, while the temperature of the other was maintained at an intermediate level (31° to 35°, usually 32° to 33°), produced a tint generally definitely more purple than that of the control Warming of one limb to a higher temperature decreased the visible colour and often gave it a slightly more orange tint Occasionally the two limbs could be put into water at very varying temperatures without any definite colour difference As a rule, however, a colour difference could be detected if the temperatures of the water in which the limbs were immersed differed by more than 8 to 12° C The warmer limb always appeared the more acid in spite of errors from any vascular dilatation which might be expected to minimise any colour difference The subdermal temperatures were close to those of the water, in one experiment where subdermal temperatures in the shaved areas were recorded by thermocouples, while the limbs were immersed in water baths which were stirred, the temperatures recorded differed from those of the baths by less than 1° C

The maximal difference in tint recorded is given in Protocol 1 The right leg at 12 5° had a colour similar to that of a tube of *p*H of 7 5, and the left in water at 40 5° was similar to that of a tube with *p*H 7 2 But the dissociation of the dye is affected by temperature, so that if these values be corrected for temperature, according to the data of Hastings and Sendroy(7) (which demonstrate that a correction of − 0 007 *p*H per 1° increase of temperature should be applied), the actual difference in *p*H would be more nearly 0 5, and the *p*H of the warmed limb would be probably in the range of 7 1 Errors already considered make the absolute value 7 1 very uncertain and of little importance, but the difference of 0 5 between the two limbs should be approximately true Very large differences may, therefore, be seen *in vivo* The smallest difference observed in experiments of this type was as follows the right leg was immersed in water at 33°, the left at 15° The right was considered to have the more purple colour, but the difference was indefinite and uncertain If, however, the colours were actually identical and temperature corrections were applied, the *p*H of the cooler limb would be at least 0 1 greater than that of the warmer

In another experiment, one leg was warmed considerably and the other cooled until a definite difference in tint was noted Both legs were then removed from the baths and were exposed wet to the room air, subdermal temperatures were determined by thermocouples and recorded

photographically No difference in colour could be detected, unless the temperature difference between the two legs exceeded 12° to 15°

It may therefore, be concluded that, in spite of the well recognised changes in the circulation rate on exposure to cold the acidity of the skin is reduced, while on warming it is increased

Acidity developed during arrest of the circulation at intermediate temperatures If the legs were immersed side by side in water at a temperature of 31° to 35° (usually 32° to 33°) and the circulation was arrested in one limb the colour gradually altered towards a yellowish tint in spite of the simultaneous development of cyanosis Protocol 1 gives an example of this in an experiment where the acidity developed was somewhat greater than that usually observed The acidity reached during stasis, and the time required on the release of the circulation to return to the initial colour varied with the duration of stasis the time values recorded were not very different from those noted for reactive hyperæmia following similar occlusions before the injection of the dye Though no exact parallelism in duration was to be observed both phenomena could often be distinguished for 5 to 10 min The absence of any exact parallelism is not significant since the condition of the animal was not necessarily the same in the two series of experiments, and in bothcases the colour differences were difficult to time exactly The reactive hyperæmia was less conspicuous in the cats than in experiments on man. Both the development and disappearance of the acid colour in the skin showed a somewhat patchy distribution the skin was everywhere acid, but the degree of acidity apparently differed in different areas

The effect of temperature on the acidity developed during circulatory arrest If one leg was immersed in cold water and the other in warm and if after some minutes the circulation was arrested in both simultaneously the warmed leg rapidly developed an increased acidity while the cooled leg showed no appreciable change in colour (see Protocol 1) In one animal a series of circulatory arrests were made at two temperatures both before and after injection of the dye and the data are presented in Protocol 2 An intensification of the reactive hyperæmia by warmth was noted (confirming the earlier observations of Lewis and Grant(8) on man), and at the higher temperature on this occasion it also lasted longer after release The acidity developed was much greater at the higher temperature, and its duration on release was also greater though the time differences were less marked than in the series with reactive hyperæmia It may be noted that the acidity developed in all the experiments was greater the higher the temperature of the bath, but that sometimes the period of recovery

on release of the circulation was not increased but decreased by the warmth (see Protocol 1, where the period appeared to be slightly longer in a bath at 35° than in one at 40 5°)

Experiments to test any possible relationship of acidity to circulatory changes Since it seemed possible that the hyperæmia produced by heat was secondary to the acidity developed, some of the ingenious experiments of Lewis and Love (9) have been repeated on these preparations These workers demonstrated that the hyperæmia produced in the lower part of one limb by immersion in water at 41° to 42°, with subsequent total immersion of the limb in water at 20° or 32°, persisted longer at the lower temperature It has been found possible to confirm their observations on decerebrate cats, and an experiment of this type on an injected animal is detailed in Protocol 2 While the persistent hyperæmia was indicated by an intensification of the colour, no increased acidity was demonstrable in the area affected If any acidity persisted, the colour change must have been so slight as to be masked by the hyperæmia Lewis and Love also demonstrated that the hyperæmia following stasis in water at 20° is of longer duration in an area which had been exposed immediately before the production of stasis to a temperature of 40° to 42° These experimental results can also be confirmed in decerebrate cats, but again no very definite increase in acidity was demonstrable in such cases

The two forelegs of an injected decerebrate cat were immersed in water at 32°, when the colour of both legs was about equivalent to a standard tube of *p*H 7 0 They were then half immersed in a bath at 41 5°, in which the immersed portion of both legs developed a colour equivalent to a standard tube of *p*H 7 4, while above the water the colour was more purple than a 7 6 standard. After 9 min. the circulation of the left leg was arrested Immediately both legs were totally immersed in water at 28° Stasis was maintained for 9½ min. In the right leg with circulation intact no definite line of demarcation due to the previous heating was visible, although the colour of the lower part of the right foreleg was somewhat more intense After 2½ min. this difference in intensity of colour had diminished until its existence was questionable In the left leg the colour gradually became less red than on the right, but no difference in either tint or colour intensity between the upper and lower part of the leg was detectable At the end of 9 min the colours were right 7 0 and the left slightly on the acid side of 7 4. On the release of the circulation the upper part of the left leg was more yellow than the lower later the lower part became more intensely coloured than the upper part and the line of previous heating was well defined. For a brief period at this time the colour below this line appeared the more yellow On repeating the experiment with an arrest of circulation in the other foreleg for 5 min., the changes were similar but the lower part of the limb, which had been previously heated, appeared to develop a slightly greater acidity than the upper

The evidence is indefinite, whether the greater acidity in the tissues developed as the result of exposure to temperatures of 41° or more can persist even after the leg has been removed to water at a lower temperature It does not seem likely that changes in acidity so difficult to detect

can entirely explain the marked hyperæmia which can be demonstrated under these circumstances In one experiment a heat hyperæmia appeared to be induced and to persist longer with later immersion at a lower temperature, when the temperature of the first bath was 40 2° on insertion of the legs and 39 4° on withdrawal 5 min later The rectal temperature was 38 7° at this time, and it is somewhat difficult to conceive that a bath at this temperature could produce an inflammatory reaction, which could account for the changes

Inflammation Since it was possible that the reaction to high temperatures was really an inflammatory response some experiments were made on other types of inflammation Some of these experiments are detailed in Protocol 2 If a number of pin-pricks were made at distances of 1 to 2 mm from one another, and the leg was immersed in warm water, no difference in colour between the affected and control areas was detectable either in tint or depth, except where the pin-hole was large, when the centre might be recognisably alkaline If a similar set of punctures was made through a $\frac{1}{300}$ solution of ergamine phosphate, a slight yellowish colour was sometimes noticed If a stronger solution ($\frac{1}{150}$) was used a yellow colour could be distinctly seen, which lasted only a short while A $\frac{1}{100}$ solution resulted in a more definite yellow colour also of brief duration. The acidity of the salt would account for it, and it is probably not significant No difference in depth of colour could be detected around these histamine punctures

If inflammation was produced by hot glass or boiling water, a marked hyperæmia developed, and usually in the centre of this area a small region of a somewhat yellowish tint could be distinguished.

Experiments on human subjects Attempts have been made on two human subjects to repeat some of these observations A solution of the dye (0 6 p c) was injected into both forearms, either subcuticularly or subdermally For the former a small amount of dye was used sufficient to infiltrate an area of 7 to 10 mm diameter to a rose-purple colour and several such areas were infiltrated in each arm. For the subdermal injections, $\frac{1}{2}$ c c of the solution was injected. Immediately after subdermal injection no colour was detectable, but within a few minutes the skin assumed a rose purple

The effect of temperature on the acidity with the circulation intact was similar to that observed in cats but much less marked. In both subjects immersion in baths differing by 25° gave a difference in tint probably corresponding to less than 0 1 *p*H of the standard tubes This difference in acidity if corrected for temperature would be probably about 0 2 *p*H, the arm in the warm bath was the more acid.

When the circulation was arrested for 20 min in baths at 42° to 39° a definite acidity was developed in both subjects, but if expressed in the same terms as those used for the cats, the change in acidity probably was not more than 0 2 *p*H even at this high temperature On release of the circulation a difference in tint suggesting acidity could be noted for 4 to 7 min , though the hyperæmia was intense for a much longer period In an experiment with a water bath at 22° the development of acidity during a stasis of 20 min was quite indefinite below the water line, but above, where the skin was warmer, a slight but definite difference could be detected Hyperæmia long outlasted any detectable acid colour

Discussion

The dye when injected into the blood stream of a cat probably passed rapidly into the tissue fluids and could be recognised soon after injection in the mesenteric lymphatics[1] The estimation of acidity was probably influenced by both the acidity of the blood and the tissues The circulatory changes observable in decerebrate cats after such procedures as temporary arrest of the circulation resembled those observed in man more closely than do those recorded for anæsthetised animals by Goldblatt(10)

The data obtained by Stadie and Martin and by Austin and his co-workers(1, 2, 3) demonstrate that human blood probably has a decrease in *p*H of about 0 02 per 1° C rise in temperature, if the total content of CO_2 *remains* unchanged The maximum change with temperature estimated in cats in our experiments, when the circulation has been intact, has been of about this order, usually the alteration has been less Possibly the increased circulation rate in the skin with diminished venosity of the blood is a compensatory mechanism The data of Schierbeck(11) also suggest that loss of CO_2 from the skin is not a negligible factor and is greater the higher the temperature

But in addition to the effect of temperature on the blood, the metabolism of the tissues is likely to be altered A diminished oxygen consumption at low temperature has been demonstrated by Goldschmidt and Light(12) The experiments involving stasis here reported indicate quite definitely that at low temperatures, not only is oxygen consumption diminished, but also acid production is reduced so as not to be measurable On the other hand, at high temperatures acid production is extremely rapid and the effect parallels that of temperature on the production of cyanosis with stasis Any compensating factors, which

[1] We are indebted to Dr R J Brocklehurst for this observation as well as for assistance in some of the experiments.

make the acid changes *in vivo* less than those observed with blood *in vitro*, must neutralise not only the changes in the physical constants in the blood (mainly an alteration in the acid constants of the blood proteins) but also the variations in acid production of the tissue Any compensation is incomplete the tissues of the skin are more alkaline when cooled and not more acid though on account of the changes in circulation rate an increased acidity might have been anticipated The end result would appear to be a balance between increases in the acidity of the blood and acid production of the tissues with a rise in temperature and a partial decrease in blood acidity through an alteration in the blood flow and loss of CO_2 Alterations of any of these factors would account for the considerable individual variation seen in different animals The question arises whether the circulatory changes are dependent upon the changes in acidity A vascular dilatation to acidity is well recognised (Leake *et al* (13)) and it is not unlikely that the vascular dilatation of reactive hyperæmia is due, either in part or entirely to acid metabolites produced during the arrest In favour of such an hypothesis is the fact that conditions which vary the intensity or duration of the reactive hyperæmia also vary the intensity and duration of the acidity The fact that a high temperature may either increase or decrease the duration of measurable acidity after an arrest of circulation, may depend on an irregular balance between the greater acid production and the increased rate of blood flow tending to remove the acid It is noteworthy that Lewis and Grant(8) reported no constant effect of temperature on the duration of reactive hyperæmia following occlusion, the effect of temperature on the duration of acidity is also indefinite and irregular The same workers demonstrated plethysmographically a much greater dilatation at the higher temperatures, the acidity developed is also greater at such temperatures

The variation in blood flow with temperature, when the circulation is intact, could be explained also on a simple variation in alkalinity if the response to temperatures above 40° to 42° be excluded At these higher temperatures a hyperæmia is induced which is preserved after immersion of the part in cold water without any definitely measurable acidity, this may best be ascribed to some other factor such as the H-substance of Lewis(14) In fact though the data reported suggest the possibility of acid being mainly concerned, in no case has the action of some other substance been excluded

A few experiments on inflammatory reactions are reported, some evidence was obtained of acid production as the result of injury, but the acidity observed does not seem adequate to account for the marked circulatory changes The acidity noted could be explained as the result

of the phosphoric acid of the histamine salt, or in the case of the scalds, as the consequence of a small local area of stasis Any fresh open wound has apparently a very alkaline reaction, but it is doubtful whether an inflammatory reaction is absent

Observations made on man indicated changes in the same direction but of much smaller magnitude It is uncertain how far the differences were due to the limitations of the technic It is possible that the acid production occurs to a large extent in the muscles and that the effect on the skin is less, owing to the greater thickness of the intervening tissues

It is suggested that there may be two chemical mechanisms through which the local circulation may be varied when the temperature is altered, one occurring at extreme temperatures when the tissues are injured and possibly due to liberation of the H-substance of Lewis, and a second utilised at all temperatures and dependent on acid-base adjustments

Conclusions

(1) Acidity changes can be demonstrated in the skin of a decerebrate cat injected intravenously with phenolsulphonephthalein

(2) The acidity varies with the temperature, a leg immersed in a warm bath shows a greater acidity than one immersed in a cold bath

(3) Acid production during an arrest of the circulation is considerable, and is greater the higher the temperature After a long arrest, the acidity may not disappear for 5 or 10 min following release of the circulation

(4) Hyperæmia produced by immersion in water at 40° to 42° and persisting when the leg is subsequently immersed in water at a lower temperature does not appear to be associated with measurable acidity

(5) The possible relation of local vascular changes to temperature and of reactive hyperæmia to changes in acidity is discussed

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PHOTOGRAPHIC METHODS OF ESTIMATING THE PERCENTAGE SATURATION OF HÆMOGLOBIN WITH VARIOUS GASES I The ratio of oxyhæmoglobin to carboxyhæmoglobin

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Introduction

The importance of being able to estimate with accuracy the ratio of oxyhæmoglobin to carboxyhæmoglobin lies in the fact that there are several purposes to which such a method can be applied amongst which are

(1) The detection of mild degrees of carbon monoxide poisoning due to leakage either of coal gas or of the products of incomplete combustion into the air which is being breathed

(2) Experimental work on the kinetics of hæmoglobin and one or both of these gases, either on blood corpuscles or on solutions of hæmoglobin

(3) The determination of the total amount of hæmoglobin in the circulating blood by the method of Douglas and Haldane(1)

The carmine method of Douglas and Haldane by which they estimated the degree to which carbon monoxide gas had replaced oxygen from its combination with hæmoglobin had the disadvantage that only a single determination could be made on one sample of solution

The reversion spectroscope of Hartridge(2, 3) did not suffer from this disadvantage since by its means a large number of determinations could be made on one sample

Both these methods suffer however from the disadvantage that neither of them obtains from the sample a permanent record which can subsequently be subjected to remeasurement and verification and both methods are liable to subjective errors

It was largely with the object of obtaining permanent records that the photographic methods to be described in this paper were investigated

The results show that the photographic method would appear to

have as wide a scope as the reversion spectroscope and to have two advantages additional to those already referred to

(*a*) The accuracy is in general higher than that attainable, even under the best conditions, by the reversion spectroscope Especially is this the case when the solution contains a high p c COHb

(*b*) The time required for making the record can (by increasing the intensity of the source of light used for photographing the solution) be reduced to a few seconds, and thus economise greatly the amount of fluid required in cases where observations have to be made on moving fluids (*e g* for purposes of kinetic studies)

The Photographic Method

Inspection of the spectrophotometric data obtained by Hartridge(2) shows (*a*) that the movement of the α band, which occurs when oxygen gas is replaced by carbon monoxide gas from its combination with hæmoglobin, is greater than the movements of the β bands or of the trough between the two bands, and (*b*) that the edges of the α band are sharper than those of the β band For these reasons our photographic records were limited to the spectrum occupied by the α band

Since the α band lies in the yellow-green part of the spectrum to which ordinary non-colour sensitive photographic plates are practically insensitive an orthochromatic or panchromatic plate had to be found that would be suitable for our purpose After testing several different makes and varieties it was found that Ilford Chromatic plates (half-plate size) were suitable

For reasons which will be discussed later it was desirable that the α band should appear on the negative as a light band bounded on either side by areas of equal blackness, *i e* should be as symmetrical as possible Reference to the absorption curves of O_2Hb and COHb shows that in both cases the area of the spectrum just to the red side of the α band is much brighter than the area of the spectrum just to the green side This lack of symmetry was approximately compensated for by means of the photographic plate which was used, for the latter being of the orthochromatic type is relatively insensitive to red light

The spectro-camera which is described in detail elsewhere(4) consisted of slit of 2 7 mm width and 7 mm length with collimator of 500 mm focal length A direct vision prism grating was designed in such a way that there was projected on to the plate a reference mark which occupies a fixed position in reference to the spectrum which is projected on to another portion of the same plate during the same exposure By means

of this reference mark it is possible subsequently to ascertain the wave length of different parts of the photographs of the spectra The camera lens also had a focal length of 500 mm The distance between the sodium lines (5893 Å.U) and the thallium line (5351 Å U) of the spectrum was 26 5 mm , *i e* the dispersion in this part of the spectrum was approximately 22 2 A U per mm

The light source employed was a fullolite lamp which was supplied with direct current at 105 volts from a set of accumulators The solutions to be photographed were placed in suitable glass cells which were placed between the light source and the slit of the spectro-camera The light also passed through a correcting colour filter (prepared by Hartridge's method) which cut off the short wave length part of the spectrum below 5300 Å U The plate in its dark slide was attached to a repeating back so that a considerable number of spectra could be recorded on the same plate

Usually photographs of spectra of solutions of known composition alternated with spectra of which the composition was the subject of determination These were taken on the same photographic plate because as is well known the sensitiveness of colour plates varies somewhat even between different plates taken from the same box

The duration of the exposure was controlled by a shutter which was interposed between the prism grating and the camera lens

The plates after exposure were developed with metol hydroquinone and were fixed, washed and dried in the usual manner

In a recent experiment successful photographs were obtained with an Ilford Chromatic plate H and D value = 135 Blood solution 1 in 200 blood, in cell 3 2 cm. thick. Exposure = 1 min. Development = 3 min

The Measurements of the Spectra

There appear to be at least three methods of performing analysis on the photographs of the spectra (A) To ascertain the density at suitable points of the plates (B) To employ the coincidence method by superposing the plate over a strip or band of the opposite density arrangement (C) To employ the coincidence method with similar parts of the same plates (or of positives taken from them) in juxtaposition

Unfortunately our own work was interrupted by other engagements at the moment when we were ready to begin analysing the plates and we have only had personal opportunity to test method (B) This method has given encouraging results, which will be referred to briefly at the end of this paper At this stage, however, it happened that Dr Hecht and

Mr Morgan were in urgent need of a photographic method of determining the ratio of oxyhæmoglobin to carboxyhæmoglobin for the accurate measurement of the dissociation curve of CO-hæmoglobin Method (A) was consequently studied by them in detail, and the particular procedure finally adopted was tested by them most thoroughly We desire to offer to them and to Mr Forbes, who continued to use the method, our warmest thanks for allowing us to describe in this section the method of analysis which has been employed by them and proved to be entirely serviceable

The densities of different parts of the plates were measured by an adaptation of the Watson densitometer This instrument consists essentially of a selenium cell, the variations of resistance of which are ascertained by means of a mirror galvanometer Two beams of light may be caused to fall one at a time on the selenium cell, one of these has passed through a chosen part of the plate which is being measured, whereas the other has passed through a standard neutral tint wedge which varies in density from one end to the other This wedge is shifted by trial and error until the light through it gives the same galvanometer deflection as the chosen part of the plate does The position of the wedge is now recorded by vernier reading and another part of the plate is selected for measurement

Fig 1 and Table I show the results obtained in a typical experiment, in which five different optical mixtures of oxyhæmoglobin and CO-hæmoglobin were photographed on the same plate Readings with the densitometer were taken at every millimetre over the part of the plate on which the α band lies, the total length of the plate thus measured being 4 to 6 mm With a later type of densitometer readings at every half millimetre have been taken

TABLE I. Densitometer vernier readings of density

Mm above reference line at which the density is read	0 p c COHb	100 p c COHb	25 p c COHb	50 p c COHb	75 p c COHb
18	Photographed as 100 p c. O_2Hb	56 2	—	—	—
19	—	54·4	—	—	59 1
20	—	53 5	63 4	58 7	57 3
21	59 7	53 8	57 9	55 8	56 1
22	52 9	55·6	54 0	54 1	56 2
23	50 2	59 0	52 2	53 7	57 6
24	50·0	—	52 0	54 3	60 1
25	50 8	—	53 3	56 2	—
26	53 1	—	55 8	59 8	—
27	57 0	—	59 9	—	—

Several different methods of determining the proportions of oxy-hæmoglobin to carboxyhæmoglobin were tested with the aid of these curves Of these two may be mentioned

(A) Direct measurement of the shift of the apex of the α band

The apex shifts about 60 A U (or 3 5 mm on the plate) when CO replaces O_2 from combination with Hb Owing, however, to the flatness

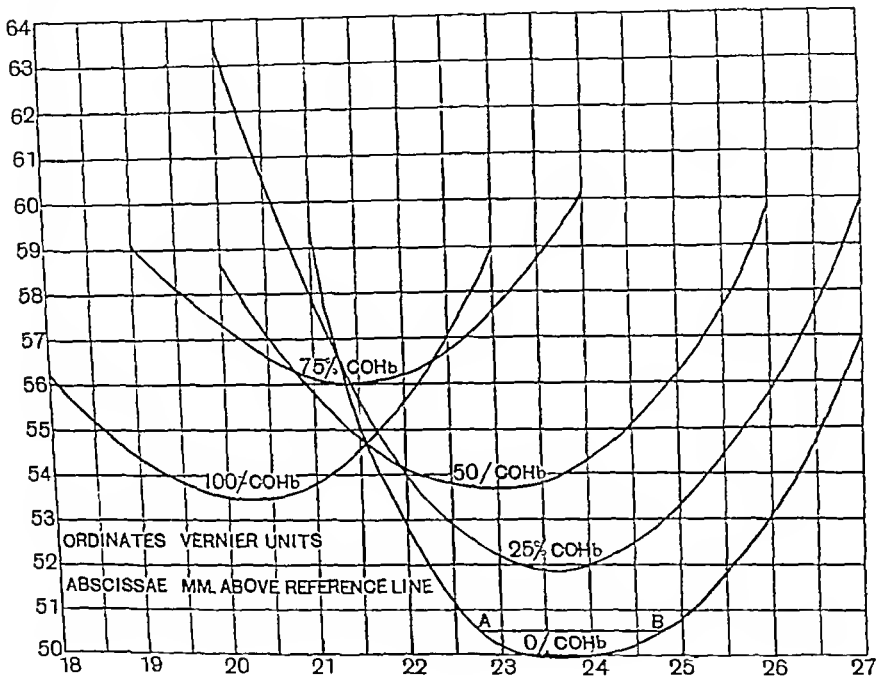

Fig 1

of the density wave length curve in the neighbourhood of the apex, the exact determination of the wave length of the apex offers very great difficulty This method, therefore, proved to be impracticable

(B) The measurement of the wave length of the mid-points of the band at a suitably selected band width

In Fig 1 the line AB drawn parallel to the abscissa axis intersects the curve for 0 p c COHb in two points A, B of equal blackness (represented by the corresponding ordinate of 50 5 vernier units) distant 22 77 mm and 24 77 mm from the reference mark respectively In this case the breadth of the band, 24 77—22 77 = 2 mm , and the mid-point of the

band at this breadth of 2 mm , is distant 23 77 mm from the reference mark

Similar results for other band widths and curves are given in Table II

TABLE II Wave length of mid point of band in mm from reference mark

Band width mm	0 p c COHb	25 p c COHb	50 p c COHb	75 p c COHb	100 p c COHb
2 0	23 77	23 66	22 92	21 45	20 21
2 5	23 84	23 65	22 92	21 43	20 20
3 0	23 87	23 66	22 92	21 42	20 18
3 5	23 92	23 67	22 92	21 40	20 16

It will be seen that the curves for 25 p c COHb and 50 p c COHb (and presumably for all intermediate mixtures) are almost perfectly symmetrical over the part of the band examined, and that the departure from symmetry outside of this range of p c COHb is only slight, and of an opposite nature at higher and low p c COHb respectively Being thus satisfied that the symmetry condition is adequately fulfilled, a single band width is selected as standard and the values of the wave length of this mid-point of each of the bands at this width is plotted against p c COHb The actual width is so selected that the slope of the curves at the extremities of the chosen width should be inclined at nearly 45°[1]

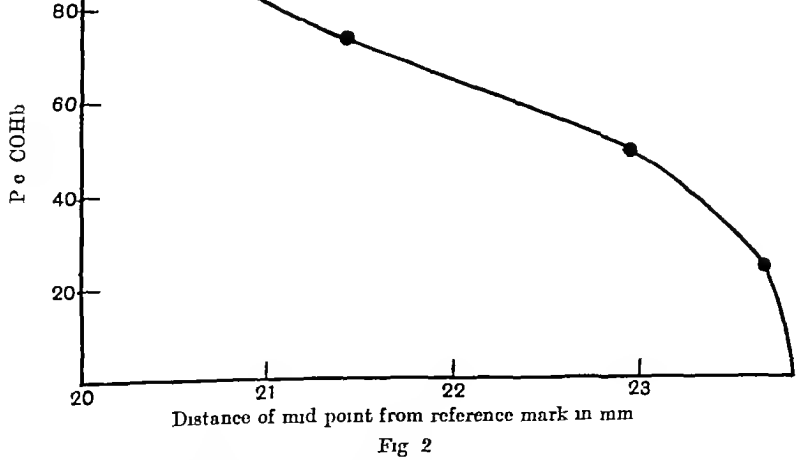

Fig 2

[1] Alternatively the average of the values of the mid point of the band for each p c COHb might have been used.

to the axis in the case of all the curves when the abscissæ and ordinates are plotted on the same relative scale as that used in Fig 1 Thus in the present example the band width of 2 5 mm is suitable The curve so obtained is shown in Fig 2, and may be described as the Calibration Curve of the plate

Accuracy of the method With careful attention to experimental details (such as the cleanliness of the solutions to be photographed) duplicate photographs of the *same* solution on the *same* plate give mid-point wave lengths which do not in extreme cases differ from one another by more than 0 033 mm., *i e* about 0 9 p c of the total shift Reference to Fig 2 shows that an error of this magnitude in photographing an unknown solution would cause an error in the estimate p c COHb which varies according to the actual range of p c COHb within which the unknown lies

TABLE III.

Range within which unknown solution lies (p c.)	Approximate error (p c.)
0–25 COHb	6
25 – 50 COHb	2
50 – 75 ,	0·6
75 – 100	1

Since individual photographic plates, even from the same batch vary in sensitivity it is necessary in order to obtain this accuracy to include on *each* plate not only the spectrum of the unknown solution, but also the spectra necessary for obtaining the calibration curve of the plate The series of p c COHb may be obtained most readily by the method of optical mixtures, but the solutions used for the latter purpose must be identical with the unknown solution in every respect except as regards the CO- and O_2-content of hæmoglobin, and the conditions of exposure, development, etc should be the same in the two cases If these requirements are adhered to the accuracy indicated in the above table is successfully achieved

It will be observed that the accuracy is very poor in the important range of 0 p c COHb to 25 p c COHb Higher accuracy in this range can however, be attained in the following manner To equal volumes of (1) 0 p c COHb solution, (2) the unknown solution add equal volumes of water containing dissolved CO,[1] so that the final p c COHb in (1) amounts to a value in the neighbourhood of 50 p c COHb Determine the actual

[1] Without allowing any of the dissolved CO to escape into the air

p c COHb in both cases (with an accuracy of 0 6 p c) The difference in the two p c values (with a small correction for the difference in the amount of dissolved CO in the two cases) gives the p c COHb in the unknown with an accuracy of $2 \times 0\ 6 = 1\ 2$ p c , instead of 6 p c

The advantages of a symmetrical wave length density curve The importance of securing a symmetrical wave length density curve has been emphasised more than once, and it is now appropriate to summarise the advantages in favour of this policy

(i) Consideration shows that the wave length of the mid-point of a symmetrical band at a selected band thickness will not be affected by small technical errors, such as slight fogging of the plate, slight inaccuracy in adjusting the strength of the hæmoglobin to be photographed, or slight errors in the time of exposure or development

(ii) Variations in the sensitivity of different parts of the same plate may amount to as much as 7 p c , but such variations are only likely to occur as between widely separate regions A symmetrical band would not be affected unless it was so wide that one side of it fell on a part of very different average sensitivity to that on which the other side of the band falls This contingency therefore is not likely to be a source of trouble in the various bands dealt with in this paper

(C) *The coincidence method by superposition*

This method is in principle identical with the one just described (B) For the plate is placed face downwards on a sheet of paper on which has been inscribed a black strip of selected width The plate is now adjusted in position by means of a micrometer until this black strip occupies the centre of the light strip on the plate which has been produced by photographing the α band The micrometer is now used to complete the adjustments so that to the eye the two parts of the band just adjacent to the edges of the black strip appear to be of equal density With suitable calibration the micrometer scale indicates the wave length of the mid-point of the band

In the previous method (*B*) two points of equal density are found where the band has a selected width by means of a density meter containing a selenium cell The wave length of the mid-point between these two points is then ascertained In this method (C), on the other hand, the eye is used for making a similar selection This method gave under test very promising results With a total difference of position of the plate of 3 72 mm between the α bands of oxy- and CO-hæmoglobin (60 Å U) the difference between the average values of ten readings on the same

band of two observers was 0 033 mm The accuracy is therefore approximately the same as that given by method (B)

This method could most probably be improved still further by bringing corresponding parts of the two opposite sides of the band into juxtaposition so that the eye could make a more direct comparison and therefore judge more readily when the two sides are of equal density This arrangement could be effected without difficulty by means of an internal reflecting right-angled prism mounted as in Brewster's pseudoscope

It may be noted in conclusion that the above method has two advantages over the densitometric method The time required for making the observations runs only into minutes instead of into hours, and the cost of the apparatus is but a small fraction of the cost of the densitometer

(D) *Coincidence method by juxtaposition*

In this method the plate is duplicated one half being placed in a reversed direction to the other half This duplication and reversal enabling the same principle to be applied to the plate as that on which Hartridge's reversion spectroscope is based

The duplication and reversal can be effected either by optical means or by cutting the plate up (or positives taken from it) This method has not been explored in any detail, but it would seem to be subject to the same limitations as those that apply to the reversion spectroscope, except that (*a*) a permanent photographic record is obtained which can be remeasured at any time, and that (*b*) the difference in colour on either side of the α band is eliminated

SUMMARY

A method is described for obtaining photographs of the absorption spectra of O_2-hæmoglobin, CO-hæmoglobin or mixtures of the two pigments The plates are analysed at leisure either by the selenium densitometer or by a visual apparatus in each case the position on the plate of the mid-point of the α band is determined to within about 0 03 mm A calibration curve relating p c COHb to position of mid-point of α band is obtained by photographing a series of known mixtures of COHb and O_2Hb, and the p c COHb in an unknown solution can be obtained by recording and analysing its photograph on the same plate as was used for the calibrating exposures The difference between the respective positions of the α band in the case of 100 p c O_2Hb and 100 p c COHb amounts to about 3 5 mm , the accuracy of the method is accordingly higher than that given by the reversion spectroscope, and has the

further advantages of giving a permanent record and of being far freer from subjective errors It is important, however, that the solution to be photographed should be free from turbidity

Our thanks are due to the Medical Research Council for defraying in part the expenses involved in this research

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PROCEEDINGS

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The electrical responses from a strip of curarised skeletal muscle under various conditions By W H Craib[1] (*University College Hospital Medical School*) (*Preliminary communication*)

Method A decerebrate frog was curarised by injecting 2 per cent curare into the dorsal lymph sac The sartorius was removed with a minimum of injury by dividing the tibial tendon, the connective tissue adhesions between it and underlying muscles, and its muscular pelvic attachment Threads were attached to pelvic end and tibial tendon It was suspended between two upright supports at a tension sufficient to render contraction isometric It was stimulated at the pelvic end by parallel electrodes so placed that the long axis of the tissue was perpendicular to the plane passing through the electrodes Thus suspended the tissue is surrounded by only a very limited layer of conducting (body) fluid. To vary the quantity of the surrounding conducting fluid a flat circular glass dish with perpendicular walls 5 mm high was placed on a movable support so that it could be gently raised beneath the suspended tissue until any required length of the latter was in contact with the surface of saline filling the dish At least 1 cm of muscle from the pelvic (stimulated) end was left surrounded by air in order to diminish the effect of the artificial stimulus on leading off contacts placed on the tissue in the dish When studying the effects of injury on the action current response, before suspending the tissue as above, the tibial end of the muscle was dipped for a few seconds into saline heated to 60° C Leading off contacts were non polarisable and pointed to a diameter of $\frac{1}{2}$ mm Responses were recorded by a string galvanometer with a string sufficiently tense to secure accuracy of deflection. Elements of the artificial stimulus were satisfactorily excluded from action current responses by (1) killing the tissue *in situ* and examining the movement of the string during stimulation, or (2) reversing the direction of the stimulating current and observing its effects on the recorded response, or (3) photographing a simultaneous record of stimulus and action current response by means of a second string To simplify interpretation of records, the tissues were examined at a temperature of about 12° C

[1] Work undertaken on behalf of the Medical Research Council.

Results (*a*) With two contacts on a sartorius suspended in air the response is diphasic, the initial phase indicating a relative negativity of the proximal contact

(*b*) With two contacts on a sartorius in contact with a quantity of saline, the response varies in form with the distance between the contacts It shows from three to six phases The initial deflection invariably indicates a relative positivity of the proximal contact

(*c*) With one contact on a sartorius immersed in a quantity of saline, and another at a distance in the surrounding medium, the response is triphasic with an initial positive phase

(*d*) With the distal contact on an injured area, and the tissue suspended in air, the response is monophasic If, without disturbing the relation between tissue and leading off contacts, the regions led off are placed in contact with a quantity of saline, the response is no longer monophasic and shows an initial positive phase

Conclusions Diphasic and monophasic responses may be obtained from a curarised sartorius under particular conditions only, *i e* when the tissue is surrounded by a very limited conducting medium They are not obtained when the tissue is surrounded by a large quantity of saline Granted that these are the facts, prevailing views on the explanation of the electrical responses of skeletal muscle appear to be called in question

The active relaxation of capillaries and venules in the reflex flare By T Lewis

In writing of the reflex flare produced in human skin by histamine, etc , I have recently expressed the view that this flare is due to the opening up of the strong (strongly muscled) arterioles, and have concluded that, so far as the minute skin vessels are concerned, any active participation in this reflex is inappreciable In stating that an active change in the minute vessels can play no more than a minor part in the flare, I have intentionally inferred that the dilatation of these vessels, which is responsible for the visible redness of the skin, is brought about mainly, perhaps entirely, as a passive effect (*The Blood Vessels of the Human Skin*, London, 1927, pp 42 and 221) With this view Krogh and Rehberg express disagreement in *Proc Physiol Soc*, Nov 12th They have attempted to ascertain the pressure prevailing in the minute venules of skin involved by vivid histamine flares, using a capsule similar

to that of Recklinghausen, and they declare the pressure to be from 6 to 11 cm of water Since they find that such pressures are little if at all greater than those existing in the same vessels when these are unflushed, Krogh and Rehberg conclude that in the histamine flare the dilated venules must be actively relaxed. If Krogh and Rehberg's values for venule pressures are correct then the conclusion that these vessels are actively dilated in the histamine flare seems to me inevitable However, I am unable to accept their values either as correct or as even approximately representing the true values They believe that the pressure applied externally by means of a transparent capsule is equal to and gauges that in the venules when "a distinct blanching ' of the skin occurs Their use of this index is eventually the result of two assumptions, first, that the index correctly gauges venule pressure in the normal skin, and, secondly, allowing this to be the case, that an identical index serves when the skin is flushed Upon the first assumption no further comment is here needed, the second assumption can be shown to be erroneous in the following way If a known pressure (say 40 mm Hg) is thrown upon the veins of the arm, it is clear that, after a sufficient lapse of time, the pressure in the minute venules will rise to at least that value If now, a pressure exactly equal to this congesting pressure, is thrown upon the congested skin by means of a capsule, this skin at once blanches, it blanches, not only "distinctly," but conspicuously, "distinct ' blanching is obtained by smaller external pressures, pressures that are known from the circumstances of the experiment to be much below the true values in the venules The amount of actual blanching seen, when equal congesting and testing pressures are used, increases progressively as these pressures are raised in successive tests In all such tests the congested skin blanches to approximately the same *end point*, namely, to its original uncongested colour (tested over the range 20 to 70 mm Hg) Thus it is abundantly clear that such an index as "distinct blanching ' is inadmissible in the case of skin flushed by congestion A truer index, and one which gives a value for the pressure within the minute vessels that cannot exceed the actual value is the pressure which, applied externally to the congested skin, reduces its colour to that of uncongested skin If this index is used in the case of histamine flares upon skin placed level with the base of the heart, values of 50 to 60 mm. Hg are obtained[1] These are minimal values for the actual pressure in

[1] If the index is ' distinct blanching' then I agree that the values are very much lower, but that is also so for the skin when the pressure in the minute vessels is raised by congesting the veins to 50 or more mm. Hg

the minute vessels, and they strongly support my idea that these vessels are dilated passively and not actively as Krogh and Rehberg believe

A full account upon which this preliminary note is based will appear in *Heart*

The effects of subcutaneous and intraperitoneal injection of oxygen upon the oxygen saturation of the arterial blood

By H W Davies and M Rabinovich

Dogs anæsthetised with luminal sodium were rendered anoxæmic by the production of pulmonary embolism in the manner described by Binger, Brow, and Branch[1] The anoxæmia was associated with considerable increase in respiratory rate which returned to normal or fell considerably when oxygen was administered by the lungs Oxygen administration by this method restored the oxygen saturation of the arterial blood to normal whereas massive injections of oxygen subcutaneously and intraperitoneally were without effect upon the respiratory rate and were associated with little or no increase in the oxygen saturation of the arterial blood A summary of these effects is given in the table It may be concluded that the use of subcutaneous and intraperitoneal injections of oxygen for the treatment of anoxæmia cannot be justified

Table I

Dog No	Weight kgm	Normal. % arterial oxygen saturation	Anoxæmic % arterial oxygen saturation	After subcutaneous O_2 injection, % arterial oxygen saturation	C c. O_2 injected subcutaneously	After intraperitoneal O_2 injection, % arterial oxygen saturation	C c. O_2 injected intraperitoneally	Breathing oxygen % arterial oxygen saturation
I	14	92	85	87	150	86	1000	—
II.	23	89	81	81	150	83	1000	94
III	13	88	78	87	200	—	—	97
V	19	87	80	85	350	82	500	95
VI.	17	86	56	43 5	300	—	—	94 5
VIII	15	85	80	80	1000	—	—	98
X	13	91 5	56	53	500	53	500	94 5

[1] *Journal of Clinical Investigation*, 1 p 155 1925

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No 1 *October* 5, 1927

No 2 *November* 21, 1927

LIST OF AUTHORS

PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY

July 2, 1927

July 23, 1927

October 15, 1927

November 12, 1927

December 10, 1927

LIST OF AUTHORS

means of which, during inspiration, 5–10 c c of the air from the end of the previous expiration is sucked over into a 250 c c sampling bottle containing air In about 20 minutes it is assumed that the air in the bottle has been replaced completely by alveolar air Cullis, Rendell and Dahl(2) have reported favourably on this procedure, but most workers, *e g* Ringer(8), Moore *et al* (7) and Rosen and White(3), have found it necessary to modify the original technique We have devised an electrical method of sampling, which is simple to operate and has given us reliable results The arrangement of the apparatus is illustrated in the figures (Figs 1 and 2) A tube leads from the space

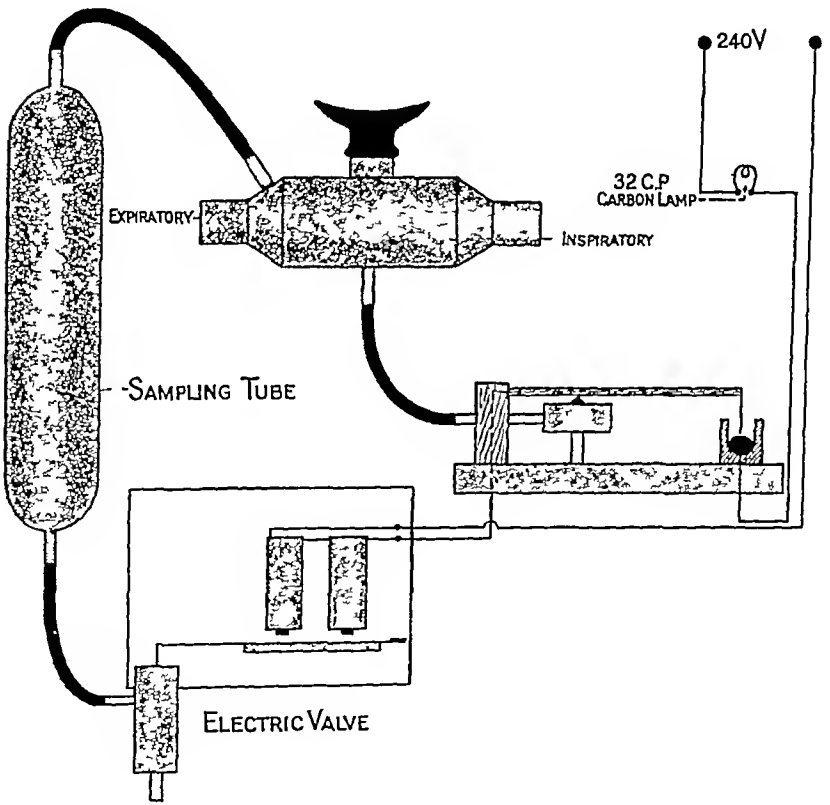

Fig 1 Arrangement of apparatus for continuous sampling of the alveolar air
I = Inspiratory valve *E* = Expiratory valve Description in text.

between the valves of the mouthpiece to a small Marey's tambour The lever which rests on the tambour dips into a mercury cup, the

height of which can be suitably adjusted The negative pressure of inspiration sucks on the tambour and thus closes the circuit of a large electromagnet, to which is attached the piston of the valve The valve is made of brass and the rod is of stainless steel The details of its construction are shown in Fig 2 There is a tube placed just beyond

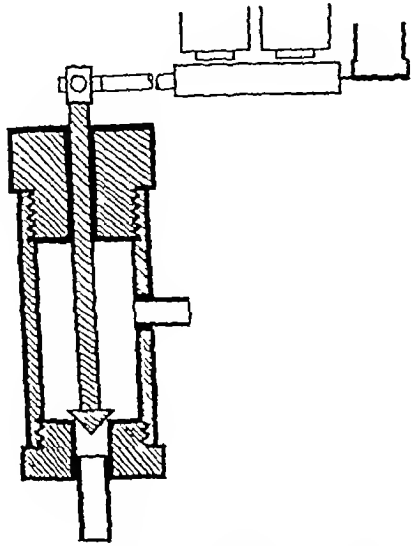

Fig 2 Detailed structure of valve.

the expiratory valve, which leads to a sampling bottle filled with fluid During each inspiration the valve piston is raised and a small amount of fluid is permitted to escape As a result, the air which is present just beyond the expiratory valve, and which thus represents the last of the previous expiration, is sucked over into the bottle The amount of air collected with each breath can be regulated by a screw clip placed on the rubber tube leading away from the valve In subjects who salivate excessively, we find that the accumulated saliva interferes with the proper working of the tambour It is advisable to interpose between the valve and the tambour a small glass "saliva trap," which also serves to prevent the full force of inspiration acting on the tambour If the lighting circuit is employed, a small carbon lamp must be introduced as a resistance By means of this valve, two 300 c c samples can be collected simultaneously in about 5 minutes

A series of experiments were carried out to compare the CO_2 content of samples collected by this method and of alveolar air collected at the end of a maximal expiration, using the Haldane-Priestley sampling

tube or the method of Dodds(10) In this latter method the subject is instructed to whistle As the note begins to fade, the nozzle of a 10 c c syringe is introduced between the lips and rapidly filled with air from the mouth This represents alveolar air We found that samples collected in this way agree with those obtained by the Haldane-Priestley technique, and samples can be obtained in this manner from subjects in which the other method proves repeatedly unsuccessful Illustrative results are shown in Table I The subject was allowed to breathe through

TABLE I

Subject	Pulmonary ventilation: Rate per minute	Pulmonary ventilation: Depth in c c.	Alveolar CO_2 p c: Before	Alveolar CO_2 p c: With valve	Alveolar CO_2 p c: After	Alveolar CO_2 p c: Difference p c	Remarks
T J	31	290	Failed	5 14	Failed	—	Untrained Over breathing
W N H.	22	340	5 86	4 89	5 33	−8	Untrained. Still over breathing after 15 minutes with valves
M K.	19	350	6 11	5 95	6 21	−4	—
B C D	21	360	5 8	4 55	4 5 4 2	+5	Over breathing into Douglas bag
M K.	16	400	—	5 6	6 3	−12	Breathe into Douglas bag
M. K.	15	470	6 8*	6 0	6 4	−6	After lunch
M. K.	13	630	—	6 36	6 03	+5	—
M K.	—	—	6 15	6 07	5 91	+2	Cotton wool resistance
S W	10	600–800	5 94	5 94	—	—	Cotton wool resistance on inspiratory side spirometer on expired side
S W	8	620	6 35†	6 34	—	—	—
S W	9	650	6-07‡	5 9‡	5 78	+2	—

* Mean of 3 † Mean of 4 ‡ Mean of 2

valves for some time before the continuous sample of alveolar air was collected The columns headed "Before" and "After" are samples of air collected at the end of maximal expiration just before and immediately after breathing through the valves It is seen from the table that if the tidal air is 600 c c or over, very close agreement is obtained between the two methods This confirms the finding of Haldane(11), that to obtain a sample of alveolar air, the depth of expiration must be 600–800 c c Most untrained subjects, however, breathe through valves in much more shallow manner We, therefore, give the subject instructions to breathe voluntarily at a slow rate, *i e* 10 to 12 times per minute As pointed out by Haldane(12), if there is no resistance to breathing, the compensation to diminished frequency by increased depth is almost perfect Adequately deep breathing is thus obtained, and very soon the subject adopts this slow rhythm unconsciously and without effort

Reference must be made to a recent communication by H. Barcroft(9) in which he has drawn attention to the older work of Krogh and Lindhard(10) These authors claim that there are wide variations in the composition of the alveolar air during each respiratory cycle, and that both the method of continuous sampling and that of Haldane and Priestley yield results which do not represent the average composition of the alveolar air to which the blood is exposed in its passage through the lungs According to Barcroft the alveolar ethyl iodide tensions obtained by the methods mentioned are lower than the true values, and consequently the circulation rate which is calculated from the use of such data may be 25 p c too high We have not yet had the opportunity to repeat Krogh and Lindhard's observations upon which the argument of Barcroft depends

II Storage of ethyl iodide

Henderson and Haggard advise that the interior of the spirometer in which ethyl iodide is volatilised should be coated with red lead, because rapid destruction occurs if ethyl iodide comes into contact with copper We have not found red lead very satisfactory A considerable fall in the concentration of ethyl iodide takes place during one or two hours Further, as a result of the interaction of the ethyl iodide possibly with the oily basis used in the manufacture of the red lead paint, a compound is formed with a very disagreeable taste and odour, so that after each experiment the interior of the spirometer must be washed out several times with fresh air before it is fit to be used again We have found that pure nitro-cellulose syrup sold under the name of luc, is more satisfactory It contains no metallic pigment, and ethyl iodide undergoes little destruction even after prolonged contact The following data illustrate this point The concentration of ethyl iodide is expressed throughout this paper in terms of the number of c c of *N*/200 sodium thiosulphate employed to combine with the iodine liberated when the ethyl iodide sample was drawn through a heated iodine pentoxide tube, 300 c c samples were usually employed

11 a.m.	Simultaneous samples	4 1 4 15 4 3, 4 25	Av 4 2
12 noon.		4 0 4 0	4 0
3 p m.	, ,	3 8 3 95, 4 05 3 9 4 05	, 3 95

Ethyl iodide may be left in the spirometer for several days, and the concentration only falls by 20–30 p c

Spirometer concentration 6 9 3 days later 4 75
" " 5·0 " 4·23

After painting the spirometer with luc, it is advisable to have it exposed to the open air for a day or two to drive off traces of the volatile solvent, which is probably amyl acetate

It is difficult to produce uniform mixing in the spirometer even when an efficient fan is employed for over half an hour Ethyl iodide is heavy and tends to sink, so that the air coming out of the spirometer first has a higher concentration than that obtained later

Spirometer reading	2	Ethyl iodide concentration	6 7, 6 6
„	„ 20	„	6 35
„	„ 40	„	„ 6 25, 6 25, 6 2, 6 25

After about 100 litres of air have been expelled from the spirometer, it is noted that the readings, though lower, are very constant

To overcome the difficulty of non-uniform mixing, we employ the electric valve described above to collect 5–10 c c sample of the inspired air during each inspiration. The "inspired air" sample consequently represents accurately the air breathed in during the experimental period We also sample the expired air in the glass mixing chamber in the same way When determining the circulation rate, we use the valve to collect simultaneously duplicate samples of the inspired, expired and alveolar air

Ethyl iodide is fairly stable in the presence of water vapour and when in contact with the surface of water, for, as we have seen above, it undergoes very slow disintegration when stored under such conditions in a spirometer An ethyl iodide mixture can be collected over water and bubbles rapidly through water without appreciable loss It undergoes very rapid destruction in a Douglas bag

Example	A	B
Concentration in spirometer	5 0	5 65
„ „ Douglas bag after 5 minutes	3 25	3 9
„ „ „ „ 20 „	—	2 15

III Estimation of ethyl iodide

We have employed the iodine pentoxide method advised by Henderson and Haggard, and use the electrically heated bath manufactured by B D H

(1) *Temperature* We find the optimum temperature for the reaction is 200–220° C

(2) *Rate of flow* We have found in conformity with other workers that the rate of air flow through the tubes of 1 litre per minute (as originally advised) gives unreliable results Satisfactory estimations can be obtained if an initial slow flow of about 100 c c per minute is used

for 3 or 4 minutes Iodine is deposited on the outlet tube, but is volatilised on running air rapidly through for another 6 minutes With an initial rapid rate of flow, ethyl iodide can be driven through two iodine pentoxide tubes in succession without undergoing complete destruction

(3) *Concentration of ethyl iodide* With concentrations of ethyl iodide equivalent to thiosulphate titrations of 8 c c or over, the iodine pentoxide method gives unreliable results, even if an initial slow flow is employed, the tube then remains unreliable for some time after, and requires re-seasoning for several hours

Experiments to illustrate the effect of rate of air current and concentration of ethyl iodide on the estimation of the latter

Ethyl iodide in air was run through an iodine pentoxide tube A, then through two KI tubes, and then into a second iodine pentoxide tube B Samples taken simultaneously from spirometer were used for each pair of estimations.

		Tube A	Tube B	Total
1	10 minutes at 1 litre per minute	5 25	0 20	5 43
2	4 minutes slow, 6 minutes fast	6·25	0 14	6 39
1	10 minutes at 700 c c per minute	4 65	0 47	5 12
2	5 minutes slow, 5 minutes at 700 c.c per minute	6 15	0·40	6·55
		First 5'	Second 5	
1	5 minutes fast (single I_2O_5 tube)	10·5	0 2	10·7
2	4 minutes slow, 6 minutes fast	—	—	15 88 12 88 14 60

(4) *Seasoning* We have used iodine pentoxide tubes prepared by the methods both of Rosen and White(3) and of Henderson and Haggard(1) Similar results can be obtained from either kind of tube, but seasoning at 250° C must be thorough and prolonged The time given by these authors for the duration of seasoning is too short, and in our experience it may take 72 hours or longer Seasoning should be continued until close agreement is obtained in the titration for two samples removed simultaneously from the spirometer Re-seasoning must be carried out at intervals For no apparent reason the tubes may cease to function satisfactorily at times and require prolonged seasoning

The iodine pentoxide method has been adversely criticised by Starr and Gamble(5), who have introduced a silver nitrate method for the estimation of ethyl iodide It must be admitted that the iodine pentoxide method is not easy to employ, but if the precautions enumerated are attended to, simultaneous samples should show agreement within 1 or

2 p c , or even less at times With prolonged use, the tubes may give lower absolute readings than previously

IV Partition coefficient of ethyl iodide, its presence in the venous blood and destruction in the tissues

A *Experiments on animals* Henderson and Haggard(1) claim that the partition coefficient of ethyl iodide between alveolar air and blood is approximately 2, and that ethyl iodide is destroyed in its passage through the tissues, so that the venous blood contains less than 10 p c of the concentration present in the arterial blood They determined the partition coefficient of ethyl iodide *in vitro* for water and blood, and *in vivo* in experiments on dogs Starr and Gamble(5) in experiments *in vitro* found the partition coefficient between air and water was 2 7, between air and blood *in vitro* the coefficient was found to be 7 6

We have endeavoured to determine the partition coefficient *in vivo* in experiments on cats and goats

In the experiments on cats, the animals were anæsthetised by intraperitoneal injection of 10–15 c c of a solution of chloralose saturated at 40° C The animal breathed in from a spirometer containing varying concentrations of ethyl iodide, ranging from 1 1–7 5 c c to 250 litres The concentration of ethyl iodide used in the determination of the clinical circulation rate is about 0 75 c c to 250 litres It is essential to use much higher concentrations in the animal experiments in order to get an appreciable amount of iodine from the samples of blood 0 5 p c CO_2 was added to the inspired air to increase the pulmonary ventilation The animal breathed through Muller valves with a very small dead space Samples of inspired air, expired air, and arterial blood from the carotid artery were analysed for their ethyl iodide content The expired air was collected in a long glass tube of about 40 c c capacity, the outer end of which just dipped under the surface of water in a basin No attempt was made to collect alveolar air, because the dead space of the smallest rubber valves we had was very large in relation to the tidal air of the cat The minute ventilation under the influence of the stimulating action of the CO_2 amounted to 360–750 c c per minute in some experiments With this degree of hyperpnœa the expired air approaches fairly closely in ethyl iodide concentration to that of the alveolar air The expired air bubbled through the water in the Muller valve before being collected in the expiratory sampling tube, but some experiments we made showed that no loss of ethyl iodide resulted from this procedure

Example Ethyl iodide mixture from spirometer was driven through dry sampling tubes, then through Müller valve into another series of sampling tubes.

	A	B
(I) Dry bottles	3 75, 3 9	4 95, 5 05
(II) After bubbling through water	3 85	5 35, 5 25

The high figure obtained in II B is probably due to the fact that a certain small amount of water containing ethyl iodide in solution was bubbled over into the sampling tube As the partition coefficient in water is over 2, the ethyl iodide content of the wet tubes may thus tend to be a little greater than that of the dry tubes.

Analysis of the blood was carried out using the apparatus figured in Henderson and Haggard's paper A stream of air is bubbled through the blood, and the ethyl iodide passes out of solution and is carried through an iodine pentoxide tube in the usual way Careful blank determinations were made previously The apparatus can deal with 5 c c of blood quite conveniently, but with quantities of 5–10 c c much care has to be exercised because a great deal of frothing takes place, and it is essential that none of the blood shall pass over into the iodine pentoxide tube We find that a run of 10 minutes, and sometimes of 15 minutes, is necessary to wash out all the ethyl iodide from the blood Samples of venous blood were taken during the course of the experiment from the saphenous vein, or at the end of the experiment from the inferior vena cava Samples of arterial blood were also taken after the animal had breathed pure air for 5–15 minutes The number of c c of *N*/200 thiosulphate used in titrating the iodine liberated from the samples of blood examined are given If the titration is under 0 5 c c it is obvious that the margin of error becomes considerable, and less reliance can be placed on the results obtained The ratio between the ethyl iodide concentration in the arterial blood and in the expired air was calculated As the ethyl iodide concentration in the alveolar air is lower than in the expired air, the true partition coefficient must be higher than the figures given The results obtained are summarised in Table II

In Exp 5, in which a mixture of 1 1 c c ethyl iodide in 250 litres air was employed, the thiosulphate titrations were very low and the results were so markedly irregular that little reliance can be placed on them

It is clear that concentrations of ethyl iodide at least 4 or 5 times above those employed clinically must be used in this work, in order to get an amount of iodine from the blood samples which bears a reasonable relation to the blood blank Considerable variations were found

TABLE II. Experiments on Cats

Expt.	Ethyl iodide in 250 L. spirometer air c.c.	Percentage CO_2 in spirometer	Concn. of ethyl iodide in 100 c.c. arterial blood during expt. (in terms of N/200 thiosulphate)	Ratio of ethyl iodide in arterial blood to that in expd. air: Average	Range	No. of determinations	Percentage return in venous blood	Remarks
1	7 5	0 5	32 3–39	3 0	2 7–3 2	2	75	After breathing fresh air for 5 mins. arterial blood still contained 45 p c. of previous ethyl iodide concentration
2	6 0	0 5	32 5–56	6 1	4 2–7 1	3	70	Arterial blood contained 35 p c. of previous ethyl iodide concentration after breathing fresh air for 15 minutes
3	5 0	0 5	20 0–41 3	4 3	3 4–5 6	4	75	—
4	5·0	—	10 3–48 0	4 4	3 5–5 4	2	40	—
5	1 1	0 5	3 7– 6 5	—	1 8–5 6	3	—	Thiosulphate titrations very low (0 15–0 75 c.c.) Results very irregular

in the ratio of ethyl iodide in arterial blood to that in expired air The average values in Exps 1 to 4 were 3 0, 6 1, 4 3, 4 4, the range being from just under 3 0 to slightly over 7 The actual partition coefficient must be higher than these figures A large return of ethyl iodide (from 40–70 p c) takes place in the venous blood Ethyl iodide was still present in the arterial blood after the animal had breathed pure air for 5–15 minutes In some of the experiments the concentration of ethyl iodide in the arterial blood increased steadily during the course of several hours, although the pulmonary ventilation showed no similar change

In the experiments that were carried out on 5 goats, the animals breathed through valves, and alveolar air was collected at the same time as the arterial blood samples A "spear-valve" respirator made by Siebe-Gorman was employed This had a small dead space, and the valves had almost no resistance whatever Owing to the very long trachea in the goat, the total dead space in our experiments was found to be high—at least 75 c c in one animal with a tidal air of about 115 c c It is therefore probable that real alveolar air was not collected and that the samples obtained represented a mixture of alveolar and some dead space air The ethyl iodide concentrations found in the sample are in consequence too high, and the real partition coefficient is greater than the figures obtained

Example

Goat 17 Kg 2 5 c c. C_2H_5 1 to 250 litres air No CO_2

3 40	Breathing started				
3 45	6 c c arterial blood	=0 56 c c	thiosulphate	=9 3 c.c	per 100 c c.
	Alveolar air	=1 54	„	=2 6	„
	Expired air	=5 55	„	=7 9	„
3 55	6 c c. arterial blood	=0 58	„	=9 5	„

Partition coefficient $\frac{9\,4}{2\,6}=3\,6$

When the inspired air contained 5 0 c c C_2H_5I in 250 litres air the arterial blood concentration was 19 4 c c thiosulphate per 100 c c and the partition coefficient was 3 4 With 10 c c ethyl iodide in 250 litres air the arterial blood contained 16 4 and 18 0 c c thiosulphate per 100 c c and the partition coefficient was 3 0

It is to be expected that in animal experiments of this character considerable variation in the results should be obtained The character of the breathing influences the degree and uniformity of ventilation of the alveoli The *p*H of the blood may perhaps affect the partition coefficient The anæsthetic may produce changes in the permeability of the alveolar epithelium, and if there is œdema of the lung, interference occurs with gaseous interchange with the blood Thus in one experiment in a goat when there was much mucus in the trachea and pulmonary œdema, a partition coefficient of 1 4 was obtained. Atropine was administered, then the experiment was repeated 30 minutes and 50 minutes later, and coefficients of 2 8, 2 7 were obtained The range of results in the goats was 2 0–3 6, the higher results being obtained in the more satisfactory experiments

These experiments of course do not tell us what the partition coefficient is in man when the ethyl iodide concentrations used clinically are inhaled for a short time

B *Return of ethyl iodide in the venous blood in man* A series of experiments were carried out to determine whether ethyl iodide returns in the venous blood in man Two methods were employed to test this point

(1) The subject breathed from the spirometer, and the expired air was collected in a glass mixing chamber of 6 litres capacity A period of 3–6 minutes was allowed to wash out the air originally present in the chamber, and then samples of alveolar and expired air were collected at 5 minute intervals for periods of 20–30 minutes If ethyl iodide returns in the venous blood, there should be a definite rise in the concentration of the vapour in the samples collected Any variation that

occurs in the extent of the pulmonary ventilation must be taken into account The results obtained are given in Table III

TABLE III.

Subject	Ethyl iodide per 300 c.c. inspired air (in terms of N/200 thiosulphate) c.c.	Preliminary breathing mins.	Time of collection of samples	Ethyl iodide per 300 c.c. expired air c.c.	Ethyl iodide per 300 c.c. alveolar air c.c.	Pulmonary ventilation per min. litres	Remarks
R W P	4 06	5	Next 5 mins	1 43	0 90	6 08	—
			,, 5 ,,	1 36	1 01	6 5	—
			,, 5 ,,	1 45	0 99	7 1	—
			,, 8 ,,	1 70	1 07	6 02	—
A.	5 89	6	,, 5 ,,	2 07	1 25	9 3	Definite rise of alveolar and expired concentrations
			,, 5 ,,	2 71	1 66	8 2	
			, 5 ,,	2 71	1 79	8 8	
			,, 5 ,,	2 83	2 05	8 3	
M K.	6 50	3	,, 5 ,,	1 65	1 11	5 3	First sample low because taken too soon
			, 5 ,,	2 64	1 51	4 7	
			,, 5 ,,	2 77	1 46	6 05	
			,, 5 ,	—	—	6 2	
			,, 6 ,,	2 63	1 83	6 35	
P	2 44	5	,, 5 ,,	1 09	—	6 9	Pulmonary ventilation diminished but expired air unchanged
			,, 5 ,,	1 01	—	6 05	
			,, 5 ,,	1 12	—	5 95	
			,, 5 ,,	1 09	—	5 65	
			,, 10 ,,	1 13	—	5 63	

In the experiment on A there was a continuous rise in the ethyl iodide concentrations in the alveolar and expired air, though the pulmonary ventilation diminished during the period of observation In the case of P, the expired air concentrations were steady though the pulmonary ventilation fell by 20 p c The other two experiments are inconclusive The ethyl iodide concentrations used in the spirometer were those used clinically or less

(2) The subject breathed for 7 to 10 minutes from the spirometer A sample of the alveolar air was collected during the last 2 minutes A series of maximal respirations were taken, and samples of alveolar air were collected at intervals Illustrative results are given below Dr Douglas informed one of us that in his experience 12 maximal respirations are sufficient to wash out a foreign gas which is present in the alveoli

Exp 1 R W P 2 5 c c ethyl iodide to 250 litres in spirometer

	Alveolar air collected	Ethyl iodide in 300 c c
7 minutes breathing from spirometer	During last 2 minutes	c c 8 3
3 ,, fresh air (13 deep breaths)	4 breaths	2 81
3 ,, ,, (12 ,)		2 10
2 ,, ,, (6 ,)	,	1 60

Duration of after breathing—8 minutes.

Exp 2. 1 25 c.c. ethyl iodide in 250 litres in spirometer

	Alveolar air collected	Ethyl iodide in 300 c c
10 minutes breathing from spirometer	During last 2 minutes	c c
3 , fresh air (24 deep breaths)	,, 6 breaths	4 75
3 , ,, (24 ,)	,, 6 ,,	2 20
3 , ,, (24 ,,)	, 6 ,,	1 05
		1 0

Duration of after breathing—9 minutes

Exp 3 0 75 c.c. ethyl iodide to 250 litres in spirometer

	Alveolar air collected	Ethyl iodide in 300 c c
10 minutes breathing from spirometer	During last 2 minutes	c.c
3 , fresh air (26 deep breaths)	,, 8 breaths	2 19
3¼ (27)	,, 9 ,,	0 70
3 , , (25 ,,)	,, 7 ,,	0·70
		0 65

Duration of after breathing—9½ minutes

It is clear from these experiments that ethyl iodide is still being given off from the mixed venous blood into the alveolar air after fresh air has been breathed deeply for periods exceeding 9 minutes If it is assumed that the samples collected during this time are in equilibrium with the arterial blood, then it follows that the latter contains considerable concentrations of ethyl iodide for many minutes after the inhalation of the vapour is concluded If the ethyl iodide concentration in the arterial blood when breathing spirometer air be taken as 100, then in Exp 1, when fresh air was breathed, the arterial blood contained 34, 25 and 20 after 3, 6 and 8 minutes respectively In Exp 2, it contained 46, 22, and 21, and in Exp 3, it contained 32, 32 and 29, after 3, 6, and 9 minutes respectively Similar results were obtained in Exps 5 and 6, quoted in the succeeding paragraph It is clear that towards the end of the period of inhalation of the mixture there was a return of at least 30–50 p c in the mixed venous blood

This agrees with the results of the animal experiments discussed earlier It should be noted that in Exps 2, 3, 5, 6, low concentrations of ethyl iodide were employed (0 5–1 25 c c in 250 litres of air)

C We carried out some experiments in which the subject breathed an ethyl iodide mixture for 15–25 minutes He then breathed fresh air, and the expired air was collected for the next 15–20 minutes, and its ethyl iodide content determined. The total amount exhaled during this period is not large, and represents 2–5 p c of the ethyl iodide previously absorbed It seems probable that the ethyl iodide which escapes from the blood into the tissues does undergo destruction there and does not

accumulate to any extent, otherwise a much larger amount would be given off in the expired air when fresh air was breathed after the experimental period Details of some illustrative experiments follow

Exp 4. R W P 0 5 c c. ethyl iodide in 250 litres, breathed for 17 *minutes* Inspired 147 5 litres (300 c c.=5 24 c c , *N*/200 thiosulphate) expired air, 300 c c =2 62 c.c , ethyl iodide absorbed=1813 c c

Fresh air now breathed 86 5 litres in 13 minutes expired air 300 c.c =0 13 c c thio sulphate Ethyl iodide exhaled=40 c c

Alveolar air after 7 minutes=0 30 c c. per 300 c c , after 12 minutes=*0 20* c c , after 17 minutes=0 08 c c

Exp 5 Pearce 0 75 c c ethyl iodide in 250 litres breathed in 23 5 minutes, 107 5 litres. C_2H_5I absorbed (3 94 per 300 c c)=*1408* c c

Fresh air, 12 deep respirations, then started collecting alveolar and expired air

C_2H_5I exhaled first 9 minutes=*22* c c

„ „ next 8 5 „ =*10* c c

C_2H_5I in alveolar air when breathing from spirometer=0 92 c c per 300 c.c

After 5 minutes of fresh air=0 17, after 17 minutes=0 10

Exp 6 Ascroft.

From spirometer, breatbed in 22 5 minutes, 136 litres C_2H_5I in inspired air=8 59 c c per 300 c c , in expired air=3 68 c c., absorbed *2226* c c

Fresh air, 12 deep respirations, then started collecting alveolar and expired air

C_2H_5I exhaled in 5 minutes =*58* c c.

„ „ next 11½ minutes=*30* c c

C_2H_5I in alveolar air when breathing from spirometer=2 54 c c per 300 c c after 5 minutes of fresh air=0 70, after next 11 5 minutes=0 35

Discussion

From the results of the experiments described above, it is clear that the partition coefficient of ethyl iodide between blood and air *in vivo* may vary from over 2 to over 7, and is thus considerably higher than that described by Henderson and Haggard We cannot say, however, what the partition coefficient is in man after breathing the clinical concentrations of ethyl iodide for a period of 20 minutes

We have also shown that there is a very considerable return of ethyl iodide in the venous blood when high concentrations are breathed, and a definite return when low concentrations are used Our results also suggest that the extent to which ethyl iodide returns in the venous blood varies with the subject, and in the same individual under different conditions Any increase in the circulation rate, general or local, *e g* in the vessels of the skin, would probably tend to cause a greater return in the venous blood

It thus appears that the fundamental propositions on which the ethyl iodide method is founded are invalid

It is claimed by some workers, however, that the method may be empirically sound and the results obtained, though not representing the absolute circulation rate, are comparable These authors claim, and we confirm this to some extent from our own experience with the method, that if the circulation rate is repeatedly determined in one subject under standard conditions, fairly uniform results are obtained This seems to us to prove very little, because we admit that probably any individual under the same circumstances deals with ethyl iodide in a uniform manner But the results would not be comparable with those obtained in the same individual under other conditions, nor are they comparable with the results obtained in other subjects who may deal with ethyl iodide in a different manner We find, too, as other workers have done, that when the circulation rate is determined by the ethyl iodide method and by the Fick principle, results of the same order of magnitude are obtained (though not without marked exceptions) As pointed out by Starr and Gamble, this agreement may be due to the return in the venous blood nullifying the effect of the high partition coefficient

The ethyl iodide method appears to be unsuitable for determinations of the output of the heart during exercise even for comparative purposes With the rapid circulation rate, the return in the mixed venous blood is even greater than it would be in the same subject at rest

We do not know in what form ethyl iodide is carried in the blood In our experiments on the partition coefficient, we observed that when air is bubbled through the blood there is a small liberation of ethyl iodide in the first few minutes, and that a much larger quantity comes off in the next 5 or 10 minutes Possibly ethyl iodide may exist in the blood in two forms, (*a*) in solution, (*b*) in some more stable state, perhaps forming a loose compound with one of the constituents of the blood

Summary

(I) An electrical method is described by means of which continuous sampling of the alveolar air can be carried out It can also be employed to sample automatically and simultaneously the inspired and expired air as well

(II) The estimation and properties of ethyl iodide are discussed

(III) The partition coefficient of ethyl iodide *in vivo* in anæsthetised animals has been shown to be between about 2 and 7

(IV) Ethyl iodide returns in the venous blood in considerable amounts

both in animal experiments and in the human subject It undergoes rapid destruction in the tissues

(V) The validity of the ethyl iodide method for determining the circulation rate is discussed

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THE REACTION BETWEEN ACETYL CHOLINE AND MUSCLE CELLS Part II

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Section I The destruction of acetyl choline by the frog's heart

In a previous paper(1) the writer described the relation between the concentration and the action of acetyl choline on the isolated heart and on the rectus abdominis of the frog Loewi and Navratil(2) have since shown that extracts of frog's tissues and particularly extracts of the frog's heart can destroy acetyl choline fairly rapidly This suggested a possible source of error in the writers' calculations both regarding the relation between concentration and action, and the amount of drug fixed by the heart, and therefore experiments were made to eliminate these errors

Experimental methods The following methods were used

(1) Immersed strip a strip of ventricle was immersed in Ringer and the isometric response was recorded

(2) Moist strip a moist strip of ventricle was suspended in air and its response recorded isometrically This method was used chiefly to estimate the amount of acetyl choline present in very small quantities of fluid

(3) Irrigated strip a strip of ventricle was arranged as above, but was irrigated by a fine jet of fluid driven by compressed air Two capillary nozzles were arranged so that irrigation either with a solution of a drug or with Ringer's fluid could be alternated rapidly

(4) Isometric ventricle the response of the whole ventricle was recorded isometrically The arrangement used is shown in Fig 1

The advantages of method (4) are that any quantity of irrigation fluid from 10 c c to 0 1 c c can be used, and can be changed quickly The whole system outside the heart was filled with boiled saline (0 65 p c NaCl) The usual initial tension was 3 cm of water, and this sufficed to produce a rapid diastolic filling The ventricle was allowed to contract isotonically except for short periods when records were taken, and thus the heart received an adequate amount of aerated fluid

In all four methods the heart was driven at a regular rate (about 15 per minute) by break induction shocks The Ringer's fluid had the

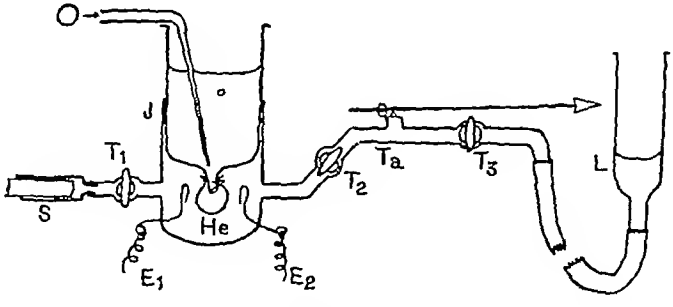

Fig 1 Apparatus for measuring action of drugs on isometric response of frog's ventricle with varying initial fillings, or varying initial tensions

He=heart, *O*=oxygen supply T_1, T_2 T_3=glass taps, E_1 E_2=electrodes, *J*=ground glass joint *Ta*=small rubber membrane tambour (diameter 5 mm), *S*=all glass syringe, graduated to 0 01 c c , for varying initial filling, *L*=movable level for varying initial tension

following composition NaCl 0 65 p c , $CaCl_2$ 0 012 p c , KCl 0 015 p c , sodium phosphate at *p*H 7 5 0 05 p c A stock concentrated solution of phosphate at the desired *p*H was used

The relation between concentration and action of acetyl choline The irrigated strip method eliminated errors due to destruction of the drug, since the heart cells were irrigated continuously with fresh solution The curve relating action and concentration obtained with this method was identical with that described in my previous paper(1), which was obtained with the immersed strip method The only difference was that a given concentration of drug produced a much greater effect with irrigation than with immersion For instance in one heart where the two methods were tested alternately, a 50 p c reduction in the force of contraction was produced by 10^{-7} molar with irrigation, whereas 10^{-6} molar was needed to produce the same effect with simple immersion. This difference depends presumably on the fact that with simple immersion the drug only diffuses slowly into the sponge-like tissue of the ventricle and as it is being broken down there continuously, the actual concentration on the cell surfaces is much less than that in the bulk of the fluid

The measurements made from strips of ventricle are, however, open to the objection that the force of contraction only represents a small fraction of the maximum force the ventricle can exert Experiments

were therefore made with the whole ventricle contracting isometrically When the initial filling was adequate, the maximum systolic tension of a fresh heart was usually 70–90 cm of water

This method was found to give satisfactory results and the curves obtained relating concentration and action were the same as those described in my previous paper(1) Fig 2 shows the results of a typical experiment

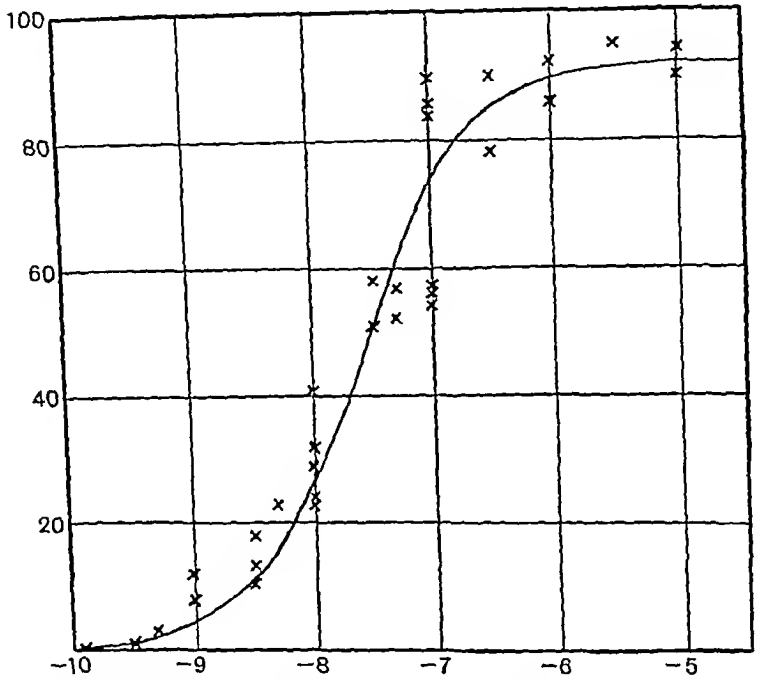

Fig 2. Action of acetyl choline on the isometric response of the frog s ventricle

Ordinate (y) = Reduction in force of contraction, expressed as percentage of normal contraction.

Abscissa = Logarithm of (x), the molar concentration of acetyl choline

The crosses show the observed figures whilst the curve is drawn to the formula $l\ x = \frac{y}{92 - y}$ ($l = 10^{7\cdot 6}$) In the figure "x" is shown on the logarithmic scale.

The results obtained with the improved methods described agree therefore with the results obtained with the simpler strip method, but the more accurate methods of recording show that in most cases acetyl choline does not produce complete arrest of the heart, for usually there

is a small residual contraction which is not abolished even when very high concentrations of the drug are employed

In my previous paper the formula adopted for interpreting the relation between concentration and action was $K\ x = \frac{y}{100-y}$ where K = constant, x = concentration and y = action produced by the drug expressed as p c of the maximum possible action this last was taken to be complete arrest The more recent methods show that the drug usually fails to produce complete arrest of the heart, however great the concentration, and therefore this formula must be modified to $K\ x = \frac{y}{A-y}$, where A = the maximum action the drug can produce, expressed as p c of complete arrest Fig 2 shows that the observations fit this formula fairly well In this case the maximum action was taken as 92 p c diminution in response

The destruction of acetyl choline by the heart When a frog's heart is placed in contact with small volumes (0 5 c c) of acetyl choline solutions it recovers rapidly from the initial effects of the drug This recovery, which is shown in Fig 3, is quickest with weak solutions and slowest with strong solutions, and is presumably due to destruction of the drug by the tissues as described by Loewi and Navratil(2) In order to estimate the rate of this destruction tests were made of the acetyl choline content of the solution, by removing small drops (0 005 c c) and applying them to a moist ventricular strip preparation (method *A*) The response of the strip was standardised by applications of acetyl choline solutions of known concentration and thus a rough estimation of the content of acetyl choline was possible The smallest concentration that could be detected by this method was about 5×10^{-7} molar The results showed that acetyl choline disappeared from the solution as the heart recovered, and that the activity of the heart was roughly proportional to the content of acetyl choline remaining in the fluid

Straub(3) described a similar type of recovery from muscarine in the hearts of aplysia, torpedo and the frog He showed firstly that in the case of aplysia the drug passed into the heart and was stored there, and secondly that the heart when it had absorbed the drug became tolerant to further applications

In the case of acetyl choline I was unable to detect any storage of the drug in the frog's heart To test this point hearts were exposed to excess of acetyl choline (10 c c of 10^{-4} molar) for periods of one to twelve hours At the end of such periods there was still a considerable concentration of drug remaining in the solution, but when the heart

was rinsed with Ringer's fluid and then ground up with sand, no acetyl choline could be detected in the emulsion.

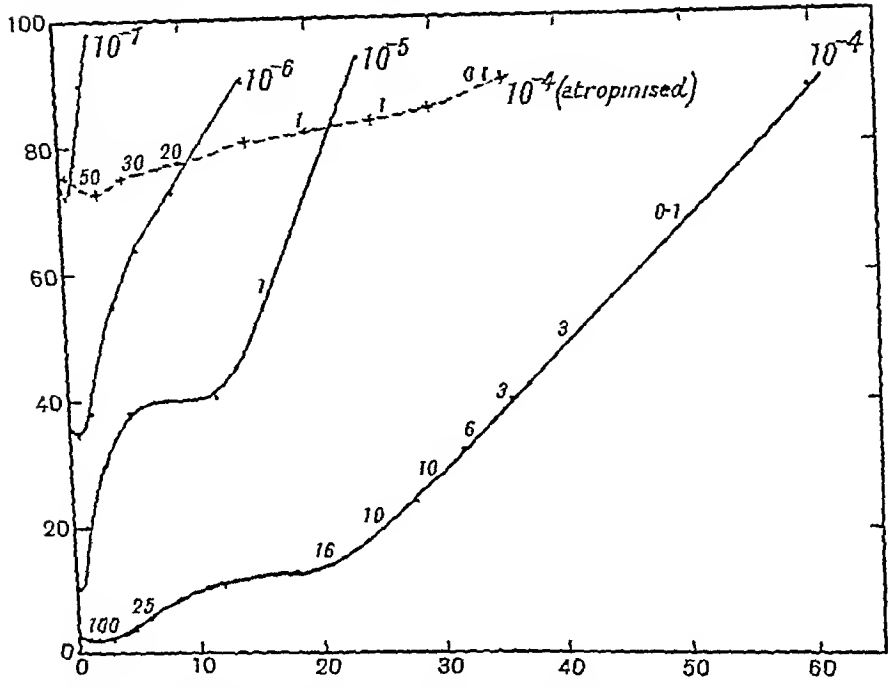

Fig 3 The destruction of acetyl choline by the frog's ventricle

Abscissa = Time in minutes.

Ordinate = Isometric contraction expressed as p.c. of normal.

In all cases quantities of 0·5 c.c. were introduced into a ventricle which weighed 100 mgm. (moist weight). The curves show the response of the heart after concentrations of acetyl choline varying from 10^{-7} to 10^{-4} molar, and also the response to 10^{-4} molar acetyl choline after the ventricle had been atropinised.

Drops of fluid were removed at intervals and their content of acetyl choline tested on a ventricular strip The figures along the curves show the molar concentrations of acetyl choline $\times 10^{7}$

The frog's heart acquires a certain amount of tolerance to acetyl choline on prolonged exposure to the drug This effect, which will be discussed later, is a source of error in the calculation of the rate of destruction of the drug but does not affect the main conclusions that the frog's heart destroys acetyl choline at a fairly rapid rate

In the experiment shown in Fig 3, 10^{-7} molar acetyl choline produced a 30 p c reduction in the force of contraction of the heart and

the times taken by the heart to recover after varying doses of acetyl choline to a response 70 p c of normal, were as follows

Initial molar concentration of acetyl choline	10^{-4}	10^{-5}	10^{-6}
Time in minutes taken to recover to 70 p c of normal response	8	19	52

The heart (which weighed 100 mgm) therefore took eight minutes to reduce the concentration in 0 5 c c from 10^{-6} to 10^{-7} molar, and therefore took $19 - 8 = 11$ minutes to reduce the concentration from 10^{-5} to 10^{-6} molar, and $52 - 19 = 33$ minutes to reduce the concentration from 10^{-4} to 10^{-5} molar From these figures it is possible to calculate the destruction in gram molecules of acetyl choline per unit weight of heart tissue per minute for a wide range of concentration

The rate of destruction is fairly uniform for any given concentration in any particular heart, for the time taken for recovery with constant concentration varies as the quantity of the drug This is shown by the following figures

Volume in c c. of 10^{-7} molar acetyl choline solution introduced	0 2	1 0	2 0
Time in minutes to reduce the concentration to 10^{-6} molar	10	45	80

A series of experiments was made in the manner described above to determine the rate of destruction of acetyl choline at varying concentrations The results obtained are shown in Table I

Table I. Time in minutes taken for reduction of molar concentration of acetyl choline All figures reduced to common standard of 0 1 c c. fluid in a ventricle weighing 100 mgm.

Date	10^{-3}–10^{-4}	10^{-4}–10^{-5}	10^{-5}–10^{-6}	10^{-6}–10^{-7}	10^{-7}–10^{-8}
16 xi. 26	—	—	—	5 7	2 8
17 xi. 26	7	6 2	3 3	4 5	2 0
25 xi. 26	—	8	3 5	2 4	1 6
9 xii 26	6	6 6	2 2	1 6	—

These figures were confirmed by other experiments made upon isolated strips of ventricle to which drops of fluid were added The rate of recovery of these strips indicated a destruction of acetyl choline at a rate of the same order as that described above The figures in Table I show that there is a considerable individual variation in the rate of destruction of the drug, but that in all cases the time required to reduce the concentration to one-tenth is three or four times greater with the highest than with the lowest concentrations This difference is remarkably small considering that the concentration varies ten thousand-fold

TABLE II

Range of molar conc. of acetyl choline	10^{-3}–10^{-4}	10^{-4}–10^{-5}	10^{-5}–10^{-6}	10^{-6}–10^{-7}	10^{-7}–10^{-8}
Gram molecules of drug destroyed per minute per mgm of heart (moist weight)	2×10^{-10} to 10^{-10}	10^{-11} to 3×10^{-12}	4×10^{-12} to 10^{-12}	6×10^{-13} to 10^{-13}	10^{-14} to 2×10^{-14}

The average figures given in Table II show that within the limits of error the log of the amount of drug destroyed plotted against the log of the concentration of the drug gives a linear relation, and the relation between the amount destroyed (x) and the concentration (c) can be expressed by the formula $K\ c^{1/n} = x$, where $K = 7 \times 10^{-8}$, and $n = 1\ 2$

The rate of destruction of acetyl choline varied considerably in different hearts, for some hearts destroyed the drug ten times as quickly as others Variations in the rate of destruction of the drug did not bear any certain relation to variations in the sensitivity of the heart One abnormal heart was found which was completely insensitive to acetyl choline even in concentrations of 10^{-4} molar, but the rate of destruction in this heart was of the same order as that in normal hearts

The destruction of acetyl choline after atropinisation is also shown in Fig 3 In this case the ventricle was exposed to atropine 10^{-5} molar, the atropine was then washed out, leaving the ventricle very insensitive, and the rate of destruction of acetyl choline was tested on a strip of ventricle The rate of destruction in this case was within the limits of variation of figures obtained with normal hearts

The fact that destruction of acetyl choline proceeds unaltered both in atropinised hearts and in hearts naturally insensitive to the drug suggests that there is no direct relation between the amount of drug destroyed and the amount of action the drug produces

Loewi and Navratil(2) showed that emulsions of heart tissue destroyed acetyl choline, and that this action was abolished by heating to 56° C They also showed that acetyl choline was destroyed by emulsions of liver and gut and to a lesser extent by emulsions of skeletal muscle I confirmed these results as regards emulsions of the heart, liver, gut and skeletal muscle and also found that frog's serum had as powerful an action in destroying acetyl choline as the frog's heart, for the destruction by 0 001 c c serum per minute was of the same order as the destruction by 1 mgm of moist ventricle per minute My experiments confirm Loewi and Navratil's conclusion that the destruction of acetyl choline is due to a ferment, and that this ferment is widely

distributed in the frog's tissues, and is not confined to those tissues on which the drug produces a specific action Many similar ferments that destroy other drugs are known For example, the frog's heart, liver and serum contain a ferment which destroys atropine (4)

The destruction of acetyl choline by the heart appears to be due to an intracellular ferment, for I found that when 0 2 c c of fluid was kept in a heart for two hours and then removed, the fluid had no power to destroy acetyl choline Moreover, the action is not due to any substance that can be washed out of the heart, for a heart that had been isolated for 24 hours and had its perfusion fluid changed at least one hundred times still retained its full power to destroy the drug

The amount of acetyl choline reacting with the frog's heart The writer has calculated (1) the amount of acetyl choline actually reacting with heart cells by comparing the effects of the drug upon a strip of frog's ventricle immersed in a large volume of solution with the effects produced when small quantities of drug are added to the moist ventricular strip This method showed conclusively that the amount of drug actually reacting with the heart cells must be very small The fact that the heart cells can destroy acetyl choline fairly rapidly further reduces the possible quantity of drug that can react with the cells

Table III shows in line 2 the figures calculated in a previous paper (1) for the amount of drug disappearing from solution when the drug acted on heart cells, and in line 3 are shown the quantities which can be attributed to destruction of the drug by the ferment action already described A comparison of the figures given in Table III shows that by this calculation the destruction of the drug would account for nearly the whole of the drug disappearing at the lowest concentration measured but that at higher concentrations the amount destroyed is only a small fraction of the quantity that disappears

TABLE III.

(1) Molar concentration of acetyl choline added	10^{-7}	10^{-6}	10^{-5}	10^{-4}
(2) Gram mols. acetyl choline disappearing per mgm of dry tissue	5×10^{-14}	6×10^{-14}	$1\,6 \times 10^{-13}$	$1\,6 \times 10^{-9}$
(3) Gram mols. of drug destroyed within 1 minute per mgm. of dry tissue	3×10^{-14}	8×10^{-13}	3×10^{-14}	3×10^{-11}

It is of course unjustifiable to assume that the whole of the drug that disappears without being destroyed by the ferment action necessarily takes part in producing the specific action of the drug A fixation or adsorption of pilocarpine and other drugs by serum and other tissues

on which the drugs exert no specific action has been described (Storm van Leeuwen(5), Beutner(6))

The figures given in my previous paper for the maximum amount of drug that can possibly react with the tissues to produce the specific action of the drug are therefore too high, and there is a considerable probability that the true figures are very much smaller

Rate of reaction and wash out of acetyl choline Experiments with isolated strips upon which a jet of solution was played made it possible to measure approximately the rate of reaction and the rate of wash out of the drug The following figures were obtained

Molar concentration acetyl choline	10^{-3}	10^{-4}	10^{-5}
Time of half action	1 4″	2 0″	3 7″
,, full action	4″	6″	9″
,, half wash out	3″	<3″	—

These figures show that the combination between acetyl choline and the tissues occurs very rapidly and that the drug can be removed equally rapidly by washing out

If the jet of acetyl choline solution be stopped, and the strip is not washed with Ringer, the heart recovers owing to the destruction of the drug This recovery due to destruction of the drug is a much slower process than washing out as is shown by the following figures

Molar concentrations of acetyl choline	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-3}
Time for half recovery on washing out	—	<3″	3″	12″	24″
Time for half recovery due to destruction of the drug	10″	12″	25″	80″	117″

The rapidity with which the action of acetyl choline is produced on introduction of the drug, and is removed by washing out, supports the view that the drug acts on the surface of the cells

Tolerance to acetyl choline Straub(3) found that when the heart of aplysia was exposed to muscarine the drug was concentrated in the heart cells, and that the heart then became tolerant to the drug He showed a similar tolerance in the frog's heart and concluded that the action of the drug depended on the difference of concentration without and within the cells Gasser and Dale(7) found that the rectus abdominis of the frog became insensitive to acetyl choline upon prolonged exposure to the drug

I observed a partial recovery of the frog's heart after exposure to a constant concentration of acetyl choline This effect is shown in Fig 4, in this case the quantity of solution employed was too great for the destruction of the drug by the heart to produce a significant alteration in the concentration The figure shows that the drug produces its

maximum effect in from 15 to 30 seconds, and that this is followed by a partial recovery which is completed within about 10 minutes This effect is seen also in Fig 3, where there is a rapid initial recovery due to tolerance, which is followed by a slower continuous recovery due to destruction of the drug

Other experiments showed that this tolerance only produced a partial recovery, and that, when a heart was exposed to the drug for periods of some hours, no further recovery occurred after the first ten minutes, provided of course that effects due to destruction of the drug were excluded

It seems unlikely that this tolerance is due to storage of the drug in the heart, because, as has been previously mentioned, no such storage can be demonstrated, and moreover is very improbable, in view of the power of the heart to destroy the drug

The full sensitivity of the heart to acetyl choline is recovered rapidly on washing out, and experiments made with rapidly moving drums showed that after washing out for even 30 seconds the full sensitivity of the heart was restored It appears unlikely that it should be possible to remove the drug from the interior of the cells at this speed

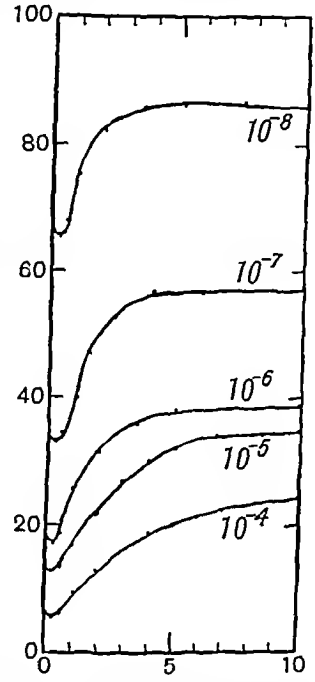

Fig 4 Response of isometric ventricle after introduction of 10 c c acetyl choline solutions of varying concentrations. Ordinate and abscissa as in Fig 3 The curves are marked to show the molar concentration of acetyl choline

A partial recovery of activity on the part of tissues exposed to a constant concentration of drug has been observed by the author in the case of other drugs acting on the frog's heart, and also has been described as a feature of the action of adrenaline on a number of tissues The phenomenon therefore is not peculiar to the case of acetyl choline I am unable to explain this tolerance effect but my experiments make it improbable that it is due to the entrance of acetyl choline into the cells, as was suggested by Straub

Another effect which I am unable to explain is that repeated applications of acetyl choline sensitise the heart to the drug This action is shown by the following figures

Time since heart isolated	20′	25′	30′	35′	120′
P c. reduction produced by 10^{-6} molar acetyl choline	72	85	92	94	94

Hearts freshly isolated were found to be less susceptible to acetyl choline than hearts after a few hours' isolation, but it was found very difficult to distinguish with certainty between effects due to prolonged isolation and those due to repeated application of the drug

In practice errors due to these causes were avoided by always ignoring the first few responses to acetyl choline given by a heart A similar sensitisation of the heart after repeated administration of a drug, or after prolonged perfusion, occurs with other drugs and therefore this effect is not peculiar to acetyl choline

Individual variations in sensitivity to acetyl choline The response of frogs' hearts to acetyl choline is characterised by a remarkable individual variation Experiments made upon 71 hearts gave the following results

TABLE IV

Molar concentration of acetyl choline needed to produce 50 p c. reduction in response		3×10^{-5}	3×10^{-5} to 3×10^{-6}	3×10^{-6} to 3×10^{-7}	3×10^{-7} to 3×10^{-8}	3×10^{-8} to 3×10^{-9}
Number of hearts	(1) Immersed ventricular strip	0	5	22	12	9
	(2) Isometric ventricle	2	0	2	11	7

In the first series the ventricular strip was immersed in the fluid and hence, for reasons already mentioned, the drug produced a more powerful action with the second than with the first method, but in both cases the figures show that the sensitivity of the hearts varies over at least a thousandfold range of concentrations

In addition to this type of variation in sensitivity to acetyl choline the hearts also varied as regards the maximum effect that could be produced by the drug In all cases the relation between concentration and action resembled that shown in Fig 2 A concentration about one hundred times that needed to produce a 50 p c reduction produced an almost maximum action, and little further effect was produced however much further the concentration of the drug was raised The maximum reduction produced was in most cases more than 90 p c of the normal beat, but in a few cases lower figures were obtained, and occasionally 60 p c reduction was the maximum effect that could be produced, even when the concentration of the drug was increased to several thousand times that sufficient to produce a reduction of 50 p c

I am unaware of any other case in which the natural susceptibility

of a tissue to a drug shows a similar range of variation As far as I could determine the sensitivity of the hearts were not influenced by the season or by the sex of the animal There are a large number of possible experimental errors that might cause apparent variation in susceptibility, but great care was taken to exclude all possible errors and the variation was found to continue unaltered

Discussion The experiments show that acetyl choline is rapidly destroyed by the frog's heart, and that this destruction must occur within or on the surface of the cells, since the ferment does not diffuse out into fluid kept in the heart The relation between the destruction of acetyl choline and its concentration follows the usual adsorption formula These facts suggest that the drug is adsorbed on the heart surface and there destroyed Experiments made to measure the amount of drug adsorbed by the heart failed to show any certain relation between the amount adsorbed and the amount destroyed, but these experiments were of necessity subject to large experimental errors

My experiments suggest that at least two independent processes occur when acetyl choline is brought in contact with tissues firstly, an adsorption and destruction of the drug by the tissues, and secondly, a reaction between the drug and certain specific receptors The latter process which produces the specific action is probably a reaction with receptors on the surface of the cells The fact that fixation of a drug by cells can proceed independently of its specific action was shown by Cook(8) in the case of methylene blue acting on the frog's heart

The action appears to be completely reversible since a similar effect can be produced and removed by washing out a hundred or more times on the same heart Nevertheless, a certain amount of irreversible change occurs for the heart's sensitivity is permanently increased by the first few applications of the drug On the other hand, when the heart is left in contact with the drug it establishes a certain degree of tolerance because the initial effect produced decreases after a few minutes These facts indicate that the factors influencing the response of the heart to acetyl choline must be complex

The curve relating the action of acetyl choline with the concentration of the drug can be explained most simply on the assumption that a freely reversible monomolecular reaction occurs between the drug and a limited number of receptors of uniform sensitivity

Gaddum(9) found that the relation between the concentration of adrenaline and its action on the rabbit's isolated uterus followed a curve similar to the one shown in Fig 2, and suggested that the relation might

be due to a frequency effect The relation cannot be explained simply in such a manner, but it can be interpreted on these lines by assuming that the drug acts on a population of receptors that vary in sensitivity, and that this variation is of an extreme "skew" type such that the distribution of receptors plotted against the logarithm of their sensitivity to the drug gives a distribution curve of the usual type This hypothesis is mentioned because it would accord with the remarkable variation in the response of individual frogs to acetyl choline shown in Table IV

Section II The influence of ions on the action of acetyl choline

It is well known that the actions both of para sympathetico-mimetic and of sympathetico-mimetic drugs are influenced by the ionic concentration of the milieu of the tissue upon which they act This fact, coupled with the resemblance between the effects of vagal stimulation and of excess of potassium, has led to numerous speculations relating vagal action with the action of ions

Unfortunately the evidence regarding the influence of ions on vagal action in the frog is conflicting Some of this confusion may be due to the fact that, as shown by Ten Cate(10), the effects produced by the sympathetic and the vagus on the frequency of the frog's heart are not influenced by ionic changes in the same way as are the effects on the force of contraction of the heart In this paper only effects upon the force of contraction will be considered

There is a fairly good agreement that the vagus is paralysed by complete lack of potassium (Ten Cate(10)), and by complete lack of calcium (Ten Cate(10), Busquet and Pachon(11), Hagan and Ormond(12)), although Brine(13) denies this latter effect Such a paralysis does not denote any specific action of these ions on the vagus because ionic changes of this extent also paralyse other nerve endings

The chief evidence regarding the effect of slighter changes in ionic concentrations is as follows

Loewi(14) and Kolm and Pick(15) state that the vagal excitability is increased when the calcium concentration is diminished but Asher(16) considers the evidence on this point to be doubtful.

Ten Cate(10) found that excess of calcium antagonised the action of the vagus, although Howell(17), Loewi(14) and Cori(18) found that this ionic change produced no certain action Zwaardemaker and Lely(19), Asher(20) and Ten Cate(21) all agree that lack of potassium first augments and finally abolishes the action of the vagus Excess of

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to wash away rapidly from the cell surfaces any carbon dioxide that was formed Ringer's fluid with a phosphate buffer was used for neutral or acid fluids and borate was used as the buffer for alkaline fluid.

Calcium concentration and action of acetyl choline Variations in calcium concentration affect the force of contraction of the frog's heart profoundly and this effect is even more apparent with isometric than with isotonic records The normal Ringer contained one millimolar calcium, and reduction of this to one-quarter reduced the response about 90 p c , whilst increase to two millimolar about doubled the force of contraction, further increase in calcium concentration produced little further increase in the force of contraction.

Table V shows that increase of the calcium content of the Ringer above normal reduces the sensitivity of the heart to acetyl choline, but that reduction of the calcium content below normal does not alter this sensitivity The response of the heart when the calcium content was below 0 5 millimolar was so feeble that it was not possible to measure accurately the effect produced by acetyl choline, but no striking change in sensitivity was noted under these conditions

TABLE V

Molar calcium content × 10^3		0·5	1	2	4
Molar conc. of acetyl choline × 10^3 required to produce 50 p c. reduction in response of frog's ventricle	(1)	8	8	—	40
	(2)	2·5	2·5	6·4	—

Although alterations in the calcium content produced considerable alterations in the sensitivity of the heart to acetyl choline yet the relation between the concentration of acetyl choline and the action produced always followed the formula given with Fig 2 and the effect of changes in calcium concentration was simply to alter the constant K

The antagonism between acetyl choline and calcium excess is in accordance with most of the observations made regarding the effect of this ionic change upon the action of vago-mimetic drugs

TABLE VI

Molar conc KCl × 10^3	0·5	1·0	2·0	4·0	8·0	12·0
Isometric response as p c. of normal	188	150	100	77	36	6

Influence of potassium on the action of acetyl choline The normal Ringer contained two millimolar potassium, and the effect of changes in this concentration are shown in Table VI These results agree fairly well with figures that the writer previously has obtained with the ventricular *strip method* (20) The effects produced by changes in the potassium concentration on the response of the heart to acetyl choline

potassium has a doubtful action Ten Cate(10, 22) and Burridge(23) state that it diminishes the vagal action, but some of Ten Cate's figures(10) suggest the reverse effect Reduction in the sodium chloride content paralyses the vagus (Witanowski(24)) Finally, Andrus(25) found that vagal stimulation produced a greater action on the tortoise heart in neutral than in alkaline (*p*H 8 0) Ringer

The evidence is equally uncertain regarding the influence of ionic changes on the action of vago-mimetic drugs

Excess of calcium antagonises muscarine on the frog's heart (Zondek(27), Loewi and Ischisaka(28)), although Loewi(14) had previously denied this, and Bouchaert(26) found that it did not affect the action of pilocarpine Excess of calcium also antagonises acetyl choline (Kolm and Pick(15) on frog's heart, Voss(29) on frog's vessels)

Bouchaert(26) found that lack of potassium inhibited the action of pilocarpine on the frog's heart, and Voss(29) found that excess of potassium augmented the action of this drug on frog's vessels

Witanowski(24) found that reduction in sodium chloride slightly reduced the action of acetyl choline on the frog's heart

Andrus(25) found that acetyl choline produced a greater action on the rabbit's auricle at a *p*H of 7 0 than at a *p*H of 8 0 The author(1) stated that the effect of acetyl choline on the frog's heart was unaltered by changes in the reaction, but this conclusion, I have since discovered, was due to a technical error, for the experimental method did not ensure that the heart cells were bathed sufficiently thoroughly with fluid to demonstrate properly the effects of changes in reaction

Voss(29) found that acetyl choline acted more powerfully on the frog's heart in alkaline solutions than in neutral solutions

This summary of results shows the variety of opinions that exist regarding the influence of ions on the action of the vagus and of vago-mimetic drugs Part of the confusion is due to the fact that the vagus, like other nerves, is paralysed by a large excess of potassium or by complete lack of calcium The effects of such extensive changes cannot therefore be compared with the effects of moderate changes in ionic content Even allowing for this fact the evidence is too conflicting to provide any certain conclusions

Experimental methods The methods described previously in this paper were used In most cases the effects of changes in ionic content were determined on the isometric ventricle of the frog, using 10 c c of fluid Ventricular strips irrigated with jets of fluid were used to determine the effect of change of reaction, since in this case it was important

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Isometric response as p c. of normal	188	150	100	77	36	6

Influence of potassium on the action of acetyl choline The normal Ringer contained two millimolar potassium, and the effect of changes in this concentration are shown in Table VI These results agree fairly well with figures that the writer previously has obtained with the ventricular strip method (30) The effects produced by changes in the potassium concentration on the response of the heart to acetyl choline

are shown in Table VII This shows that reduction in the potassium concentration increased the sensitivity of the heart to acetyl choline whilst increase in the potassium concentration decreased this sensitivity

TABLE VII

Molar content of potassium $\times 10^3$	0 5	1 0	2 0	4 0
Molar conc of acetyl choline ($\times 10^5$) which produced 50 p c reduction in response	1	3	5	8

Increase in the potassium chloride content above 0 004 molar produced so great a reduction in the force of contraction that it was difficult to measure the effect of acetyl choline, but no striking change in sensitivity to acetyl choline was observed with these higher concentrations of potassium The fact that decrease in potassium increases the action of acetyl choline accords with the results of various workers who have shown that this change increases the sensitivity of the frog's heart to vagal stimulation

The influence of reaction on the response to acetyl choline Changes in hydrogen ion concentration produced a very marked effect on the isometric response of the whole ventricle The carbon dioxide produced by the heart cells was a possible source of error since it tended to alter the reaction of the fluid in contact with the cells, therefore experiments were also made with ventricular strips irrigated with a jet of fluid The two types of experiments gave concordant results and typical figures are shown in Table VIII

TABLE VIII

Hydrogen ion concentration $\times 10^9$	0 1	1 0	6	30	60	300	600
Isometric response in cm. water	10	60	40	28	19	10	2

Experiments both with the *strip* method and with the whole ventricle showed that decrease in the hydrogen ion concentration antagonised the action of acetyl choline Typical results are shown in Table IX

TABLE IX.

Hydrogen ion concentration	10^{-7}	$1 6 \times 10^{-8}$	10^{-9}
Molar concentration of acetyl choline $\times 10^5$ producing 50 p c inhibition	2	3 2	8

Accurate results could not be obtained with solutions with a *cH* above 10^{-7} because the heart beat feebly in such solutions, but the experiments showed that acidity did not alter the action of acetyl choline to any striking extent

These results confirm the conclusions of Andrus (25) (acetyl choline on rabbit's auricle) but are opposed to those of Voss (29) (acetyl choline on frog's heart)

The figures given above do not show adequately the full changes produced by alkalinity on the response to acetyl choline Not only does the heart become less sensitive as measured by the concentration needed to produce 50 p c of maximum inhibition, but also the maximum inhibition that can be produced by acetyl choline is reduced

This difference is shown clearly in Fig 5, which shows that when

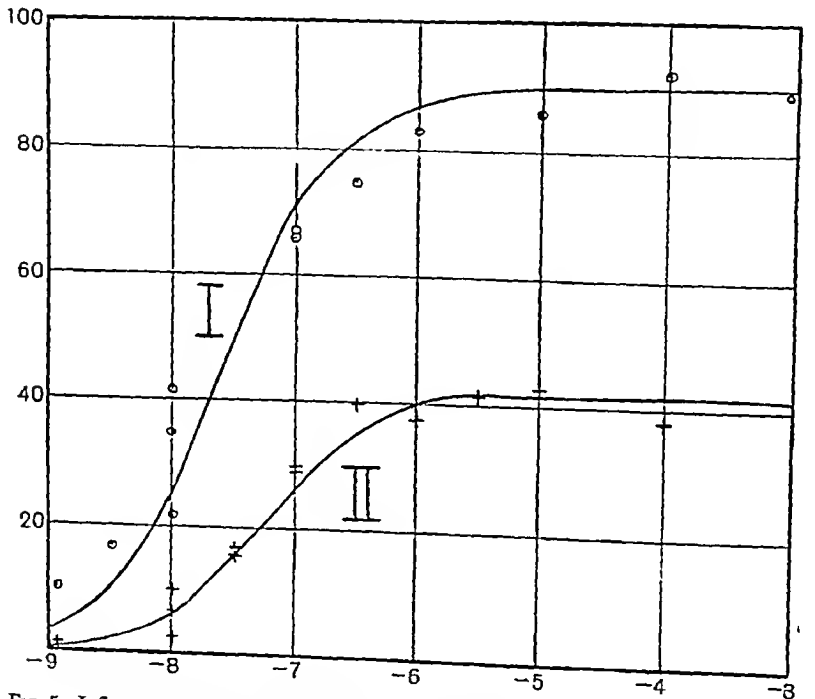

Fig 5 Influence of reaction on the action of acetyl choline

Ordinate and abscissa as in Fig 2

The crosses show the figures observed, whilst the curves were drawn to the following formulae. (x and y as in Fig 2.)

Curve I. Phosphate buffer pH=7 5 Formula I $x=\frac{y}{90-y}$ ($I=40\,000{,}000$)

Curve II Borate buffer pH=9·0 Formula I $x=\frac{y}{42-y}$ ($I=20{,}000\,000$)

the heart was perfused with Ringer pH 7 5 the maximum effect produced by acetyl choline was a reduction of 90 p c in the response, but that when the pH was 9 0 acetyl choline only produced a 40 p c reduction in the response

Figures from other experiments showing the same effect are given in Table X

TABLE X

Hydrogen ion concentration	Maximum reduction in response produced by acetyl choline					
	(1)	(2)	(3)	(4)	(5)	Average
10^{-7} to 3×10^{-8}	100	95	90	95	—	95
1.6×10^{-8} to 6×10^{-9}	65	92	50	90	90	77
10^{-9}	—	80	50	—	42	57

Increased alkalinity therefore makes a certain proportion of the heart cells completely immune to acetyl choline

Discussion My results show that the intensity of action of acetyl choline on the frog's heart is increased by reduction in the potassium content, and that it is reduced by increased potassium or calcium content or by increased alkalinity As far as could be ascertained no marked effect was produced by decrease of calcium or by increased acidity

Since acetyl choline is destroyed by the frog's heart, experiments were made to determine whether ionic changes altered the rate of destruction of the drug, since such alterations would be a possible cause for changes in the sensitivity of the heart to the drug These experiments however all gave negative results

The effects produced by ionic changes on the action of acetyl choline are of course of particular interest if we accept the hypothesis of Loewi(2) that stimulation of the vagus causes the release of acetyl choline, since in this case the changes observed in the action of acetyl choline should throw light on the relation between vagal action and the action of ions The antagonism of acetyl choline by excess of calcium and by increased alkalinity is in accordance with the majority of observations regarding the effect of these changes on vagal action These effects are moreover such as would be anticipated because excess calcium and increased alkalinity produce effects on the frog's heart almost exactly the opposite of those produced by either acetyl choline or by vagal stimulation The effects produced by alterations in the potassium content on the action of acetyl choline are much more difficult to understand

Diminution of potassium concentration definitely increased the action of acetyl choline, and several observers have shown that this ionic change also increases the action of the vagus Increase in potassium content antagonised the action of acetyl choline, but the evidence regarding the influence of this change on the vagus is indecisive

These effects are the opposite of those that might have been anticipated. In the first place excess of potassium depresses the contractility of the heart in a manner very similar to vagal stimulation, whilst lack of potassium produces a systolic effect somewhat resembling sympathetic stimulation. In the second place calcium and potassium are supposed to be antagonists and yet variations in these two ions produce a similar effect on the response to acetyl choline or to vagal stimulation. Calcium and potassium are therefore not antagonists as regards their effects on the response of the heart to acetyl choline. Other evidence is available showing that calcium is an imperfect antagonist of potassium For example, calcium lack and potassium excess produce different changes in the electrical response of the frog's heart (Daly and Clark(31)) and on conduction in the tortoise auricle (Seliskar(32)) Moreover changes in the content of one ion can only be antagonised as regards their action on contractility over a fairly small range of concentrations (Clark(9))

The effects produced by ionic changes on the response of the heart to acetyl choline and to vagal stimulation can be explained on the hypothesis that these latter actions are dependent upon the perfusion out from the heart of potassium. Howell and Duke(33) showed that vagal stimulation liberated potassium from the mammalian heart This was denied by Hemmeter(34) who worked with elasmobranch hearts, but was confirmed by Asher(35) who used the frog's heart.

The passage out of potassium from the heart should be antagonised by excess of potassium in the Ringer and should be favoured by lack of potassium The writer(36) has shown that perfusion of the frog's heart with potassium free Ringer results in a loss of potassium from the cells

On the other hand, this loss of potassium would be antagonised by any change that made the cell wall less permeable, and excess of calcium and increased alkalinity are believed to produce this effect Hence this hypothesis would explain why the two last mentioned ionic changes antagonise acetyl choline Unfortunately this hypothesis is very difficult to reconcile with the fundamental fact that lack of calcium, excess of potassium and acetyl choline all produce a very similar depression of the contractility of the frog s heart

My experiments support Ten Cate's(10) conclusion that the action of the vagus or of vago-mimetic drugs cannot be identified with the action of potassium in any simple manner, but that nevertheless there appears to be some special connection between vagal action and the distribution of potassium in the heart cells and in the surrounding fluids

Conclusions

Part I

1 The relation between the concentration of acetyl choline and its action on the frog's heart has been tested with a variety of experimental methods and in all cases the same relation has been found as was described in a former paper

2 The destruction of acetyl choline by the frog's heart resembles a ferment action and the relation between the amount destroyed in unit time by unit weight of tissue (x) and the concentration (c), over a range of concentrations from 10^{-3} to 10^{-8} molar, is given by the formula $K\ c^{1/n} = x$ ($K = 7 \times 10^{-8}$ and $n = 1\ 2$)

3 Acetyl choline combines with or can be washed out of the heart in a few seconds, and the rate of action and rate of wash-out are of a similar order

4 The presence of acetyl choline cannot be demonstrated within heart cells after prolonged exposure to the drug

5 The amount of acetyl choline that produces the specific action of the drug is probably considerably smaller than the maximum amounts previously calculated

6 The individual susceptibility of frogs' hearts to acetyl choline varies over a remarkably wide range

Part II

The action of acetyl choline on the frog's heart is modified by changes in the ionic content of the Ringer's solution

The chief changes produced are as follows

The action of acetyl choline is reduced by increased calcium or potassium content or by increased alkalinity

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FURTHER OBSERVATIONS ON THE REACTION OF SMOOTH MUSCLE TO THE H-ION CONCENTRATION

By B A McSWINEY and W H NEWTON

(*From the Department of Physiology, University of Manchester*)

In a previous paper(1) the effect of changes of *p*H upon smooth muscle capable of sustained variation in length was described and the length of the muscle was shown to be dependent on the *p*H of the surrounding fluid for any given period Differences in direction and sensitivity of the response at different ranges of the *p*H scale were also demonstrated In this paper experiments are described which deal with the effects of similar changes of *p*H on strips of smooth muscle which normally exhibit rhythmic activity with little or no tonus mechanism

Farndon(2) in 1908 stated that with the mammalian uterus preparation alkali augmented tonus with diminution of spontaneous contractions and death in contraction Acid on the contrary lowered the tone of the muscle, with diminution of spontaneous contractions, sudden strong acidity causing death in forcible contraction Young(3), in 1914, found that HCl up to 0 006 p c caused relaxation of the muscle of the small intestine as did also CO_2, but as phosphates were present in the solution it is difficult to calculate the *p*H corresponding to the strength of the solution. 0 5 p c HCl was found to abolish all movements Botazzi(4) in 1916 stated that the tone of isolated intestine was increased by all alkalies and diminished with acid Evans and Underhill(5), in 1923, showed that the effect of a small increase in the *p*H was to augment the frequency of contractions while a decrease in the *p*H caused a diminution in the rate

Method Strips of muscle mainly from the region of the pylorus and lower body of the cat's stomach were used in these experiments The regions proximal to the antrum, while exhibiting rhythmic movement were also capable of permanent change in length, and were therefore unsuitable for a study of effects dealing primarily with alteration of rhythm The strips of muscle were suspended in Ringer-Tyrode solution in the glass muscle chamber Phosphates were omitted, as in previous experiments,

to obviate precipitation in alkaline solution and also to have sodium bicarbonate as the sole buffer To control the *p*H of the solution, O_2 and CO_2 were bubbled in by the methods previously described For large alterations of *p*H sodium hydroxide and hydrochloric acid were used The temperature of the fluid was controlled at 37° C by a thermostat

Experimental results The effect of a moderate fall in *p*H was to cause a decrease in the frequency of the spontaneous contractions of the muscle from the pyloric antrum with no observable change of the base line (Fig 1) Occasionally this result was accompanied by a diminution in height of the contractions, often, however, the contractions showed a preliminary increase in size which was, in many experiments, sustained A greater decrease in the *p*H caused a cessation of the rhythmic contractions and the tracing became a straight line

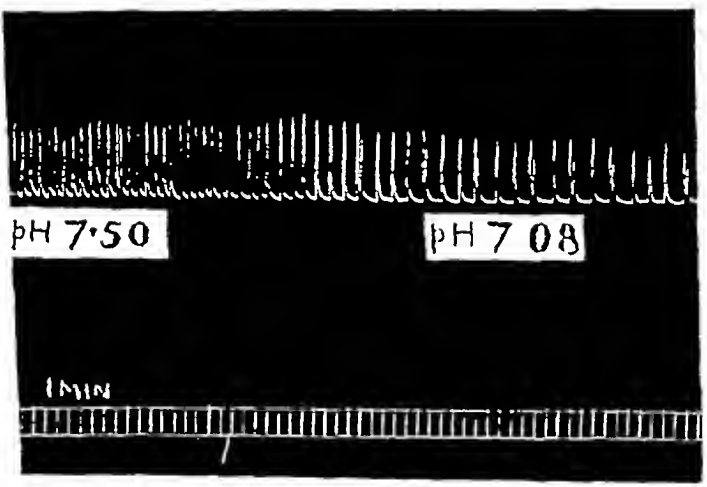

Fig 1 Antrum of cat. Tracing to show decrease of rate of contractions on decrease of *p*H with onset of spasm Time tracing, one minute

A moderate increase in *p*H caused acceleration of the contractions which were often increased in height If the *p*H of the solution was raised above *p*H 8 the amplitude of the movements tended to become smaller though the rate of contractions showed a further increase No alteration in the base line was observed throughout this range

In most experiments an interesting type of contraction was observed when the rhythm of movement was depressed by increasing the acidity Two or more contractions became grouped together, the lever making

an incomplete return to the base line between phases The groups of contractions occurred quite regularly and, in tracings taken from preparations of antrum, replaced the single contractions This spasm effect is seen in Fig 1, being initiated as the *p*H is lowered In Fig 2 the effect may also be observed passing off as the *p*H is raised Spasm usually occurred in the region of *p*H 7 1

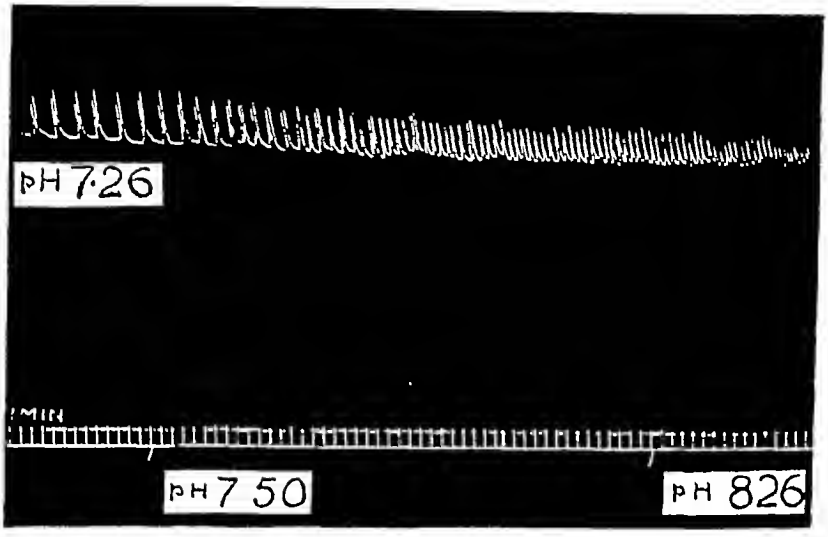

Fig 2 Circular preparation of cat's antrum Tracing to show augmentation of rate of contractions with disappearance of spasm on increase of *p*H Time tracing, one minute

In regions of the stomach other than the antrum, *e g* lower body, similar effects were observed in the main, but the results were complicated by the simultaneous occurrence of tonic changes, accompanied by irregularities in the contractions similar to those described in our last paper Almost invariably a decrease in frequency was accompanied by an increase in amplitude and vice versa The final abolition of contractions, by increase in acidity, was preceded by spasm, but like the contractions themselves, this stage was more irregular than that seen in the antrum (Figs 3 and 4) An initial spasm was often seen on lowering the *p*H and once or twice violent spasm occurred for no apparent reason It may be recalled that, in the fundic region of the greater curvature of the cat, a peculiar tonus effect was observed on lowering the *p*H, a cycle of relaxation and contraction preceding the main relaxation In the present series of experiments the base line, instead of falling when the *p*H was diminished,

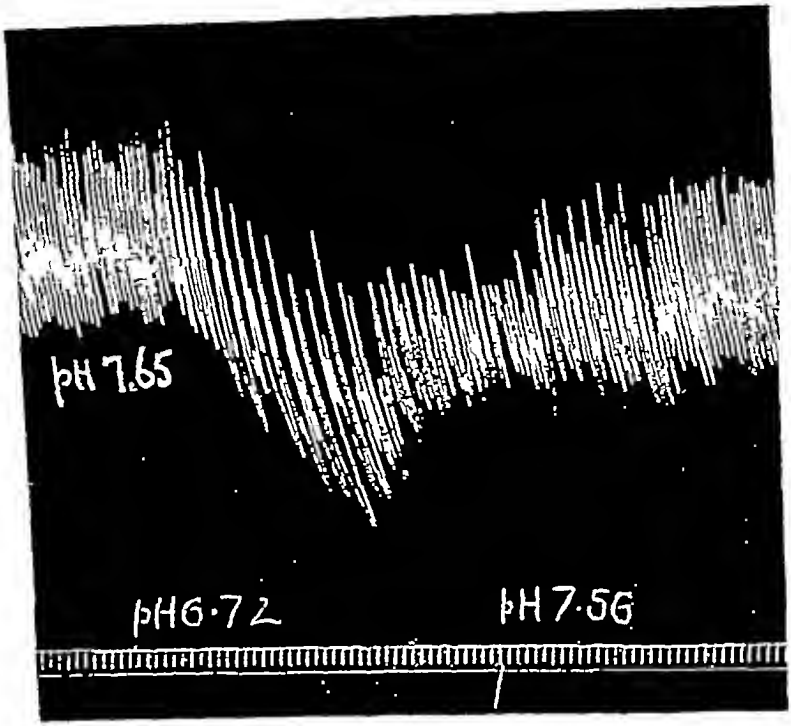

Fig 3 Preparation of slmg of cat. Tracing to show effect on the length of the muscle and rate of contractions of alterations of *p*H. Time tracing one minute.

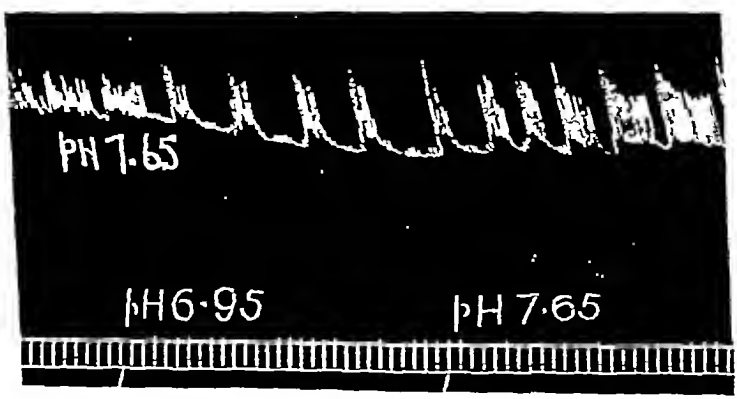

Fig 4 Upper body of cat Tracing to show onset and disappearance of spasm. Time tracing, one minute.

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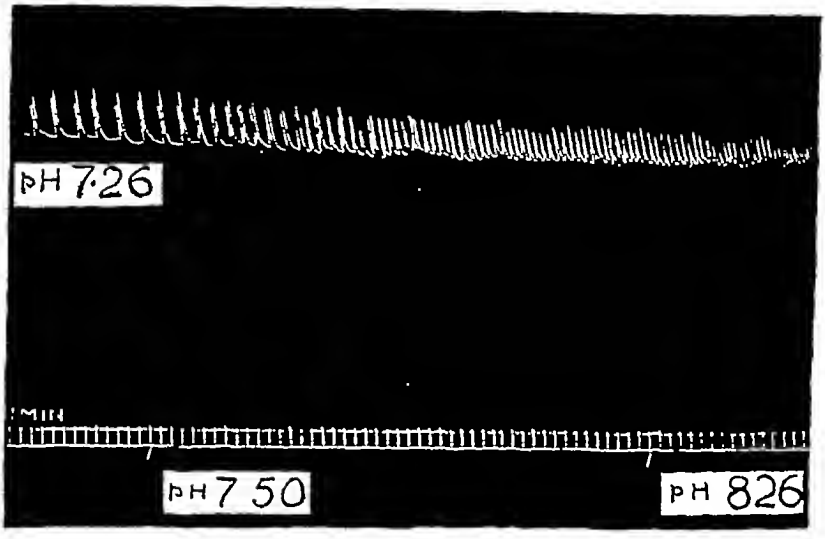

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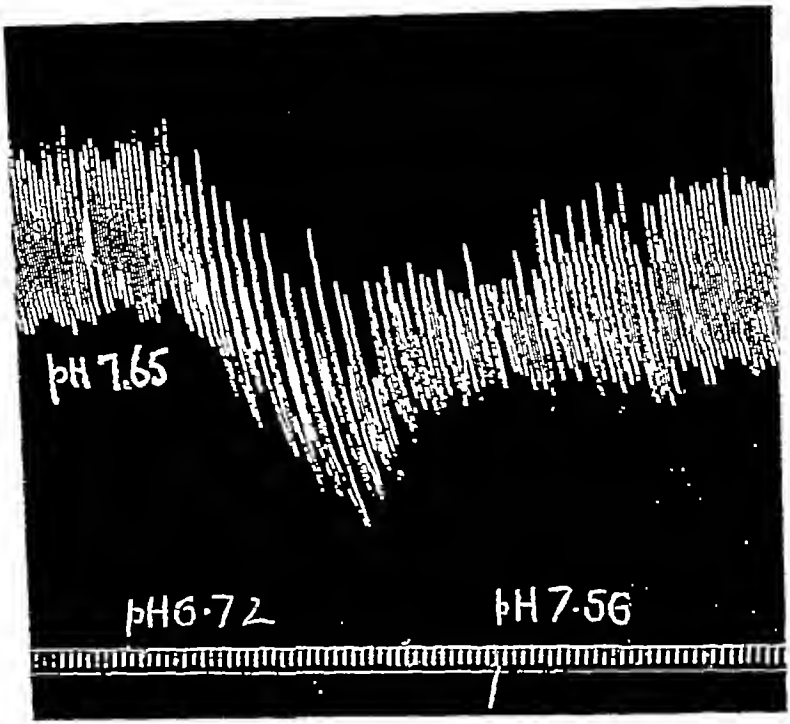

Fig 3 Preparation of sling of cat. Tracing to show effect on the length of the muscle and rate of contractions of alterations of *p*H. Time tracing one minute.

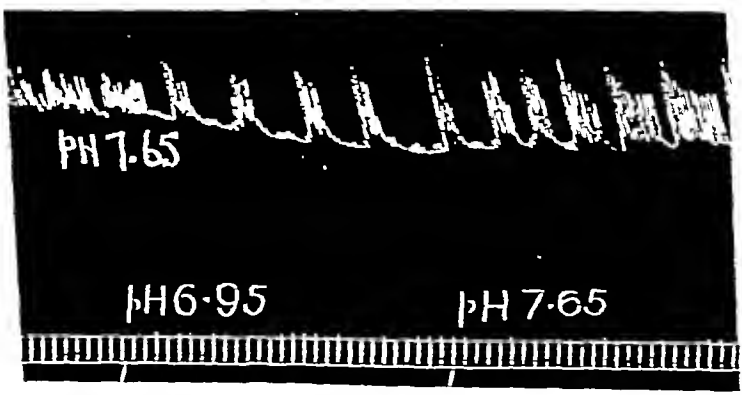

Fig 4. Upper body of cat. Tracing to show onset and disappearance of spasm. Time tracing one minute.

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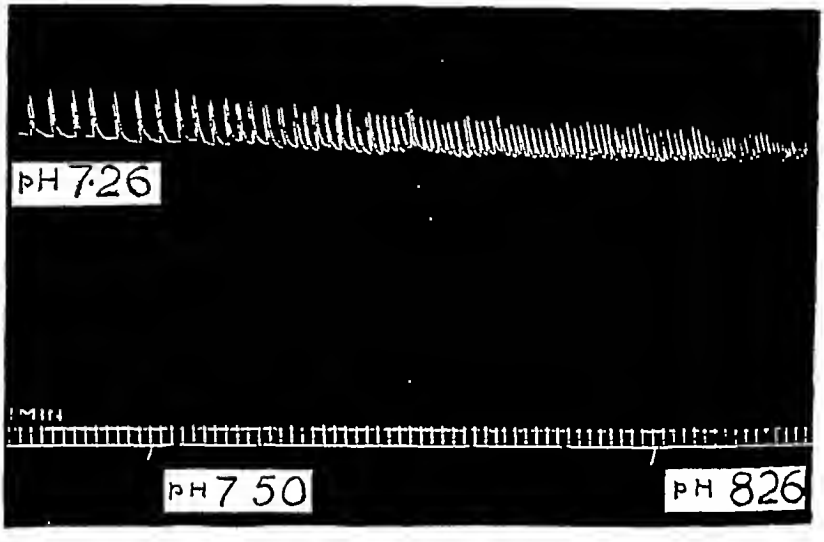

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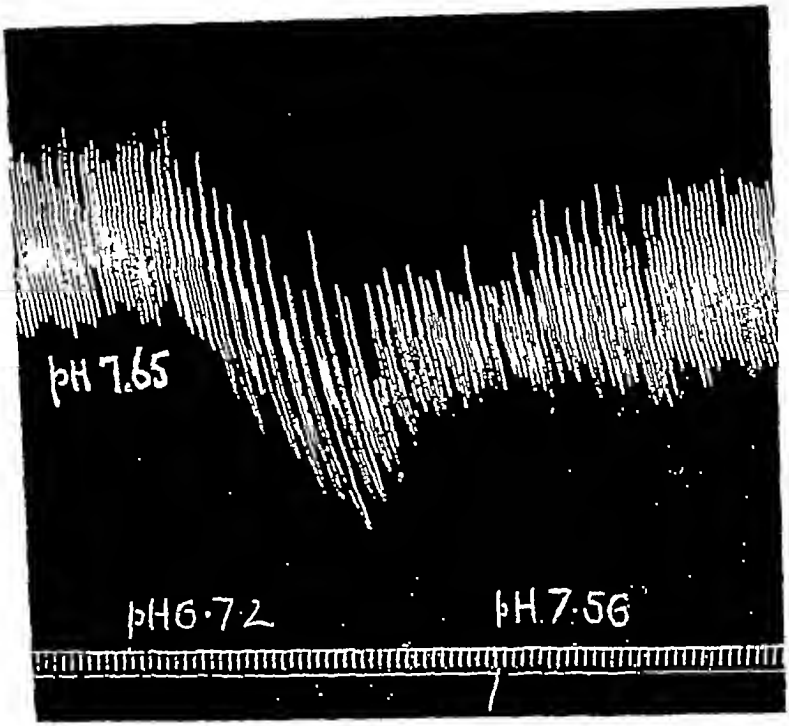

Fig 3 Preparation of sling of cat. Tracing to show effect on the length of the muscle and rate of contractions of alterations of *p*H. Time tracing, one minute.

Fig 4 Upper body of cat Tracing to show onset and disappearance of spasm. Time tracing, one minute.

rose steadily and fairly rapidly for five or six minutes, while the rate of contractions was well maintained After this initial effect the base line began to fall in the usual way, the frequency of contractions becoming much diminished with an accompanying increase in the amplitude

In the description of our experimental results we have sharply divided the antrum from the remainder of the stomach in order to separate the simple antral type of change from the more complicated reactions of the proximal regions There is, however, a gradual transition from one part to another, as even from circular and longitudinal strips of the fundus, tracings have been obtained which show a fair degree of

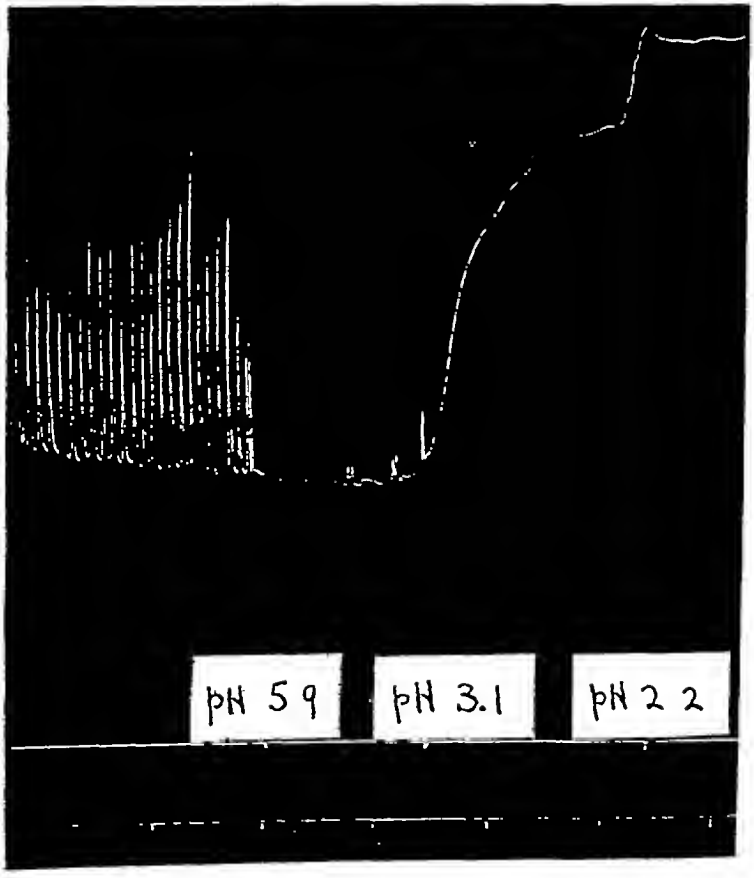

Fig 5 Antrum of cat. Tracing to show response of muscle to large decrease of *p*H. Time tracing, ten minutes.

regularity in the rate and height of contractions The general rule is that as the fundus is approached, the rhythm becomes gradually more irregular and the muscle fibres are more prone to alter in length as the *p*H is varied. This is accompanied by a greatly increased frequency of spontaneous contractions, in contrast to the slow regular pulsations of the antrum.

When the muscle has been stimulated by pilocarpine, the effects of *p*H changes upon the rhythm can still be superimposed Examination of the tracings shows acceleration on decreasing the acidity, retardation on increasing the acidity, spasm and total cessation can also be observed The effects are, however, damped by the initial treatment of the muscle and delicate transition effects are difficult to obtain.

The reaction of rhythmic strips of the antrum to large changes in *p*H are interesting As in previous experiments, the action of the strong acid was ascertained by adding to the 250 c c of Ringer-Tyrode solution, which bathed the muscle, successive doses of 0 125 c c of 30 p c HCl The first addition of acid caused a complete cessation of all spontaneous contractions the second, a rise in the base line the third, a further rise in the base line while subsequent doses have no effect except possibly a fall of base line

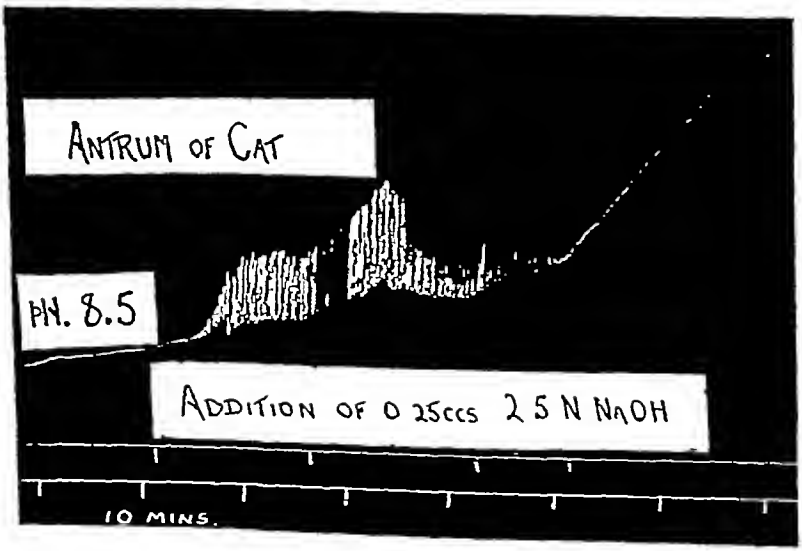

Fig 6 Antrum of cat. Tracing to show response of muscle to large increase of *p*H. Time tracing, ten minutes.

In order to obtain any response of the tissue on addition of 2 5 N NaOH, beyond an alteration in the rate of contractions, it was found necessary to cut off the stream of CO_2, to prevent the formation of bicarbonate The solution has, therefore, an initial pH of 8 5–9 0, the oxygen bubbling being continued throughout the experiment In experiments in which the initial value of the H-ion concentration of the solution was regulated to a value of pH 7 5 by the use of CO_2, both gases being left bubbling, the rhythmic contractions were increased in frequency, but the strip showed no alteration in length, even when the total alkali in the solution equalled $N/20$

If the bubbling of CO_2 was arrested the addition of 0 25 c c of 2 5 N NaOH, first stimulated then depressed the spontaneous contractions The stimulation was sufficiently great to initiate contractions in strips of quiescent antral muscle (Fig 6) A rise of base line accompanied the rhythmic changes but was not marked until a concentration of $N/100$ NaOH had been reached (1 c c of 2 5 N NaOH to 250 c c of solution) At this point, a marked sustained contraction always occurred, usually with cessation of rhythmic contractions Further addition of alkali failed to bring about the end relaxation such as was obtained in the muscle of rabbit fundus

Discussion

We are able to divide smooth muscle into three divisions according to its movements and tonus (1) muscle capable of only change in length; (2) muscle showing rhythmic activity, (3) muscle capable of changes in length and rhythmic movement These three types of muscle can be demonstrated in the stomach

In a previous set of experiments we showed that the first type of muscle responded to changes of pH in a manner similar to the swelling of gelatine on changing the H-ion concentrations In these experiments the effect of alterations of pH on the rhythmic type of smooth muscle has been observed We find that moderate decrease of pH decreases the rate and amplitude of movement, but causes no change of base line Addition of strong acid ultimately brings about contraction of the muscle The response of the muscle to alterations of pH in the opposite direction are similar, moderate increase causes increase in rate of contraction but no change of length, further addition of strong alkali, however, brings about a contraction of the muscle

On decreasing the pH a grouping of contractions was frequently observed in the region of pH 7 1 We have termed the condition "spasm"

owing to the similarity of the tracings to those obtained by McCrea and McSwiney(6) These observers in recording the movements of the pyloric antrum on stimulation of the peripheral end of the vagus nerve found that "a strong stimulus may cause a sustained contraction, the rhythmic movements increasing in rate and starting above the base line indicating summation" The tracings illustrating this statement are similar to those obtained in these experiments It is of interest that stimulation of the vagus with a strong current can bring about a similar condition to that obtained on alteration of the H-ion concentration

The reaction of the antral muscle to changes in H-ion concentration may be summarised as (1) alterations of rhythm to moderate changes, (2) alteration in length to extreme changes If we compare these results with those obtained on the fundus, we find that the sustained contraction occurs on the acid side at the same point *p*H 5 9 and at a comparable point on the alkaline side

Changes in length of the fundus muscle by small alterations in *p*H to either side of *p*H 7 5 correspond to the alterations in frequency of contractions found in the tracings taken from strips of the pyloric antrum The contractions obtained by addition of strong acid and alkali appear to be similar in all types of tissue These findings are not in entire agreement with the results of Evans and Underhill who found that smooth muscle always relaxed on the addition of acid but in their experiments a muscle possessing considerable tonus was used which would correspond to a strip from the lower body of the stomach

We do not wish to suggest that these results are any indication of a lactic acid mechanism in smooth muscle, as the response of the tissues under the conditions of experiments appears to us to be of the nature of a protein reaction, similar to the swelling of gelatine, as we have previously pointed out The variation of frequency to alteration in H-ion concentration occurs in exactly the same range as the reversible change in length recorded in "tonus" muscle The concentrations at which the sustained contraction takes place are also comparable The different type of response occurring in strips of smooth muscle taken from different situations suggests that the tissues possess different buffering properties and this property may be related to function. The reaction of the fundic muscle (tonus muscle) to alterations in *p*H is a graded reaction throughout, while in pylorus muscle the comparable protein reaction is represented by the end contraction which occurs outside physiological limits

To test this hypothesis, the reaction was studied in other preparations, whose structure contains, at the most, only a small amount of smooth

muscle The tracing in Fig 7 illustrates the effect of changes in H-ion concentration on a strip of human skin In this tissue a reaction only

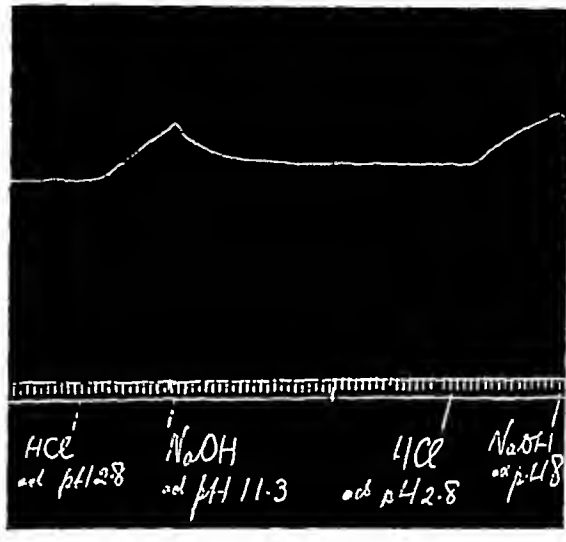

Fig 7 Strip of skin. Tracing to show effect on tissue of changes of *p*H. Time tracing, one minute

occurred with large changes of *p*H, the response was, however, similar in direction to the results obtained with strips of smooth muscle under similar conditions An attempt was made to demonstrate a pure protein effect on a strip of gelatine suspended in cold solution (Fig 8) A change of length was obtained on addition of strong acid and strong alkali as with skin, but the reaction was opposite in direction, relaxation on decrease and contraction on increase of *p*H The change in length occurs at a H-ion concentration, at which, according to Loeb(7), gelatine increases in volume The reaction may be explained on the suggestion that the increase in the size of the gelatine, under the conditions of tension, would be represented on the tracing by an increase in length, whereas in the muscle prism, swelling of the cell, resulting in an increase of pressure, would cause a bulging of the lateral wall and a shortening of the whole element

In view of the suggestion that the reaction of smooth muscle to alterations in H-ion concentration is of a protein nature, a recent paper by Gorter and Grendel(8) is of interest These observers demonstrated the effect of H-ion concentration on the thickness of a protein film At

the iso-electric point and at a *p*H 1 or 2, a very thin film is obtained, but at both sides of the iso-electric point the thickness is far greater

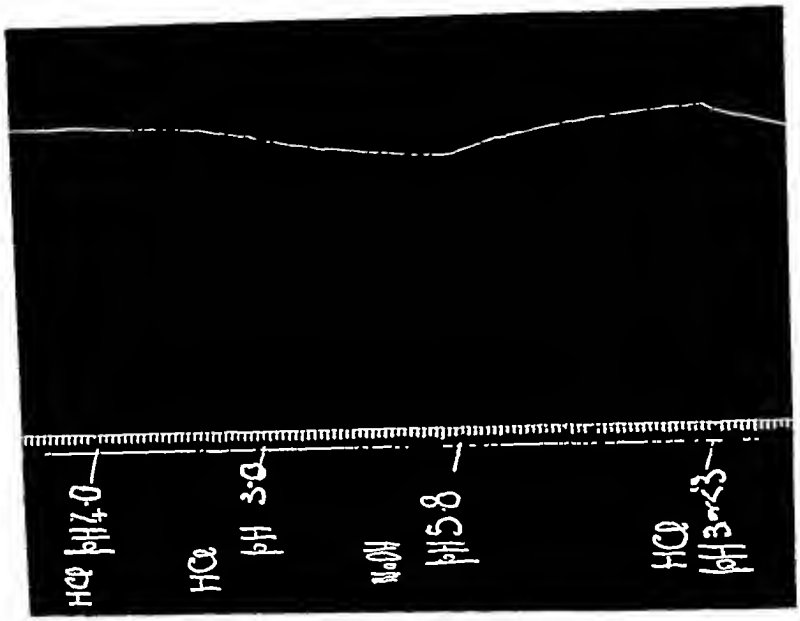

Fig 8 Strip of gelatine. Tracing to show change of length on alteration of *p*H. Time tracing, one minute

The thickness of these films is often about three times greater than the monomolecular The curve showing the relation of the *p*H to the thickness of a casein film, is similar to the curve we have previously published

Conclusions

1 The reaction of rhythmic contracting smooth muscle to changes in the H-ion concentration is analogous to the reaction of "tonus" muscle

2 Alterations in frequency of contractions, augmentation on increasing, and diminution on decreasing the H-ion concentration, within narrow limits, are analogous to the changes in length observed in tonus muscle

3 Sustained contraction of both types of muscle was observed with either a large increase or decrease of H-ion concentration at comparable points

4 A grouping of contractions (spasm) was observed on decreasing the *p*H

The expenses of this research have been defrayed, in part, by a grant from the Govern ment Grant Committee of the Royal Society

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VASCULAR PROPERTIES OF TRAUMATISED AND LAKED BLOODS

By D B PHEMISTER and J HANDY

(*From the Institute of Physiology, University College, London*)

Attempts to demonstrate the presence of a vaso-dilator substance in the venous blood collected from the limb during the period of reactive hyperæmia following obstruction of its circulation have led to observations of vaso-dilator action of shed blood which form the basis for this report One of the most plausible theories for the explanation of reactive hyperæmia, advanced by Anrep(1) and supported by Lewis and Grant(2) and Goldblatt(3), is that a vaso-dilator metabolite is formed in the tissues of the limb during the period of vascular obstruction, and that this causes relaxation of the capillaries and arterioles Such a substance would, after release of the obstruction, be either destroyed *in situ* or carried away by the circulating blood.

Dr G V Anrep, at whose incentive the work was begun, suggested that circulation of the venous blood collected from the limb during the period of reactive hyperæmia might produce vaso-dilatation, and devised a vivi-perfusion apparatus for that purpose

Description of the apparatus The apparatus consists of a flask attached to a cannular system which can be inserted in the course of the main artery of the limb (Fig 1) By adjusting the T-bore tap (*S*) the blood may be sent directly from the proximal into the distal limb of the cannula or it may be diverted through the flask (*F*) passing in at one end and out at the other by way of the connecting tubes (*C* and *D*) For purposes of filling or emptying, the flask is provided with extra outlets at the top and bottom. Before emptying the flask the outlet tube *D* is obstructed by turning its stopcock, or, in the absence of a stopcock, by the application of a clamp A tube for registering the arterial pressure connects the distal limb of the cannula with a mercury manometer The bore of the glass tubing leading to the flask is 4 mm. and that of the glass cannulæ which are inserted in the artery is the largest that can be introduced, averaging 2½ mm. for the femoral artery of the dog Flasks varying in size from 30 c c to 75 c c were used.

They were of Dewar construction in order to reduce the loss of heat from the blood.

Dogs weighing preferably 10 to 15 kg were used, 0 3 to 0 5 c c of 10 p c morphine acetate was given hypodermically In the early experiments anæsthesia was induced by ether and continued by chloralose, 0 075 grm per kg body weight, given intravenously This frequently produced slow and deep respirations which disturbed the plethysmographic tracings Urethane 0 3 grm per kg was then used in place of chloralose with improved results but respiratory difficulty was sometimes encountered Finally ether was given alone by means of an automatic inhalation apparatus and with its use the most uniformly satisfactory tracings were obtained A 3-inch incision with its middle point at Poupart's ligament is made over the vessels of the left lower limb The upper part of the femoral artery and the lower part of the iliac artery are exposed and all of their branches ligatured and divided The peritoneum is retracted upward and the posterior iliac artery ligatured The branches of the femoral vein are then tied off and severed The limb is denervated by division between ligatures for hæmostasis of the anterior crural and sciatic nerves and of the branches of the lumbar plexus along the iliac vessels 0 35 to 0 4 grm of heparin is then given intravenously to prevent coagulation of the blood. A Y-cannula is inserted into the femoral vein and the cannulæ of the vivi-perfusion apparatus into the femoral artery (In later experiments where arterial blood only was used no cannula was put into the vein) The flask and T-bore stopcock are supported by clamps from a ring stand Connections are then established for recording blood-pressures in the carotid artery and in the cannula of the femoral artery A 5-second time marker is used

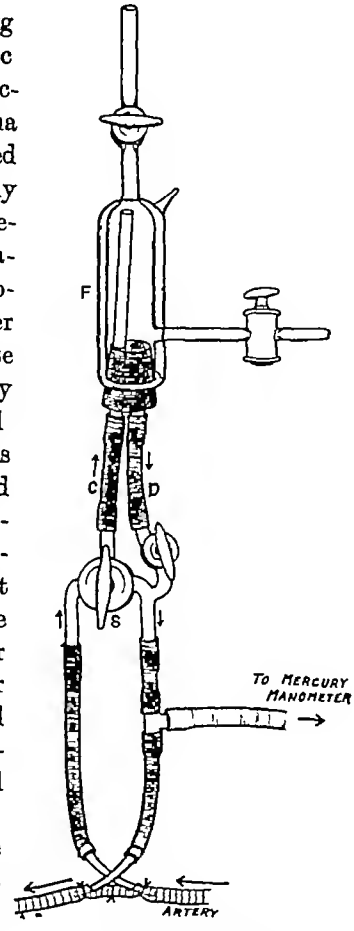

Fig 1 Anrep's vivi perfusion apparatus.

A plethysmograph is applied to the limb and filled with water at a temperature of 35° Electric light bulbs or heaters are applied alongside the flask and plethysmograph to maintain constant temperatures and the animal's temperature is kept normal by the use of blankets and by the application of heat externally when necessary

Under favourable conditions the experiment thus set up may be carried on over a period of 4 to 6 hours before clotting occurs or the vessels lose their power to respond. Altogether 92 experiments have been performed with this apparatus and its modifications, with varying degrees of success in 69

Results of experiments.

Obstructions of the artery were interspersed with the vivi-perfusion experiments in order to test the hyperæmia reactions (Fig 3 *a*) In general they were found to correspond with those reported by Goldblatt A 30 c c flask was used regularly, except when specified 30 c c of blood were collected from the femoral vein immediately after the release of obstructions of the femoral artery varying from 1 to 5 minutes in duration and transferred to the flask. It was circulated in the limb 1 to 5 minutes after recovery from the hyperæmia and regularly produced vaso-dilatation as evidenced by increase in limb volume and decrease in limb pressure lasting over a period of 40 to 60 seconds Samples of blood were then collected from the femoral vein after recovery of the limb from the hyperæmia and from the jugular vein, transferred to the flask and circulated in the limb They were found to produce as marked temporary increase in limb volume and decrease in limb pressure as did the blood collected from the femoral vein during the period of hyperæmia No special care was taken against traumatism of the blood during these experiments Arterial blood was stagnated in the flask for 1 to 10 minutes by adjusting the T-bore tap and then circulated in the limb (Fig 2 *a*) It normally produced no vaso-dilatation and when vaso-dilatation occurred it was less than that produced by the venous bloods

It was then considered probable that there is normally a metabolite in venous blood capable of producing vaso-dilatation when re-circulated Since the most marked difference between arterial and venous bloods is found in their gaseous contents, it was thought that decrease in oxygen might be responsible for the reaction. Consequently the effects were determined of both arterial and venous bloods whose gaseous contents were modified outside the body Blood was placed in tonometers which

were then exhausted by a suction pump for 10 to 15 minutes during shaking In some instances the blood was then exposed to varying

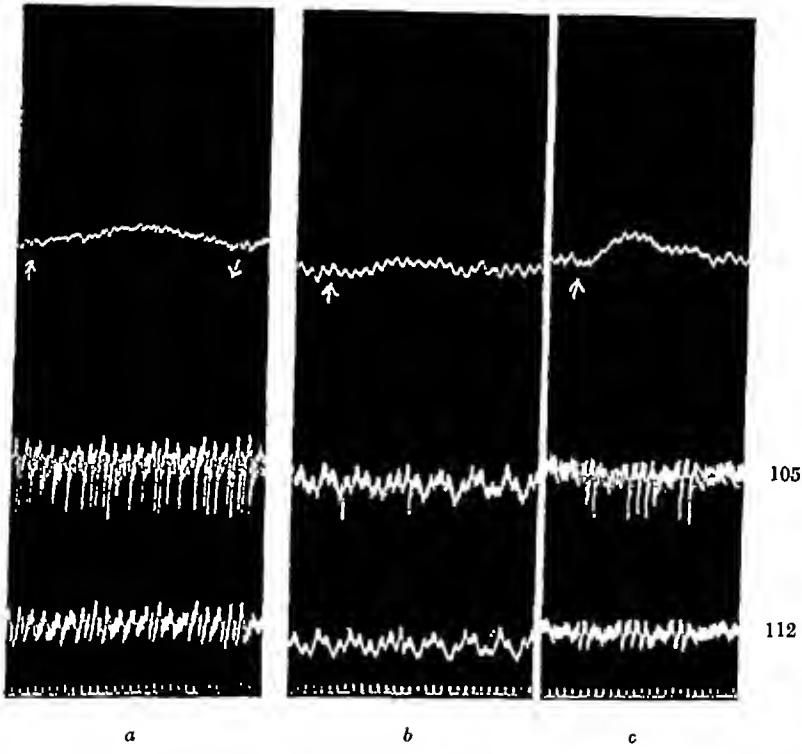

Fig 2, Exp 15 *a* Circulation of arterial blood stagnant in short-circuited flask for 4 minutes. *b* Circulation of jugular vein withdrawn under hood without shaking *c* Circulation of arterial blood removed under hood without shaking

tensions of CO_2 at 37° and atmospheric pressure The CO_2 content of the blood was varied in these experiments from minimal values to the largest obtainable by this method Exhausted blood was also circulated In all cases, however, and quite independently of all changes made in the blood gases either arterial or venous blood always gave a conspicuous vaso-dilatation when circulated in the limb

Since traumatism of the blood was a constant factor in all experiments in which the gaseous contents were modified, it was decided to test the influence of traumatism alone Arterial blood was withdrawn, shaken by hand for varying lengths of time, returned to the flask and circulated It regularly produced vaso-dilatation (Fig 3 *c*), the degree

usually varying directly with the duration of the shaking up to a certain point Active shaking for 10 seconds caused a definite though small

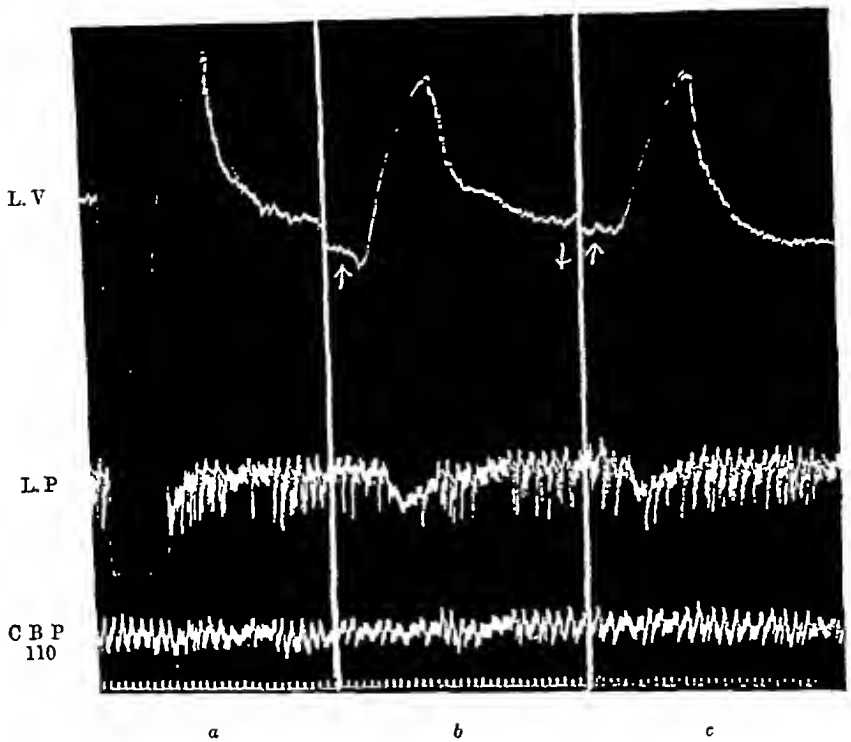

Fig 3 Exp 15 *a* Reactive hyperæmia following obstruction of femoral artery *b* Circulation of blood from jugular vein shaken 1 minute *c* Circulation of blood from femoral artery shaken 1 minute

reaction The reaction increased with prolongation of the period of shaking up to 2 or 3 minutes, when a maximum effect was produced This corresponded roughly with the length of the obstruction necessary to produce the maximum hyperæmic reaction in the same experiment Further traumatism for as long as 10 minutes produced no additional effect Shaking with beads or with a funnel inverted in the beaker into which the blood was withdrawn hastened the rapidity with which the vaso-dilator property developed Samples of venous blood were subjected to traumatism and were found to produce the same reactions as similarly treated arterial blood (Fig 3 *b*) In contrast with these experiments it was found that when either arterial or venous

blood was withdrawn for a few minutes and introduced into the flask with care and not traumatised while outside, it usually produced either slight or no vaso-dilator action when circulated in the limb (Fig 2 *b* and *c*)

In order to determine more accurately the influence of traumatism in the production of the vaso-dilatation, it was decided to traumatise the blood without removal from the flask A glass bead was inserted in the flask before the start of the experiment In the course of the experiment blood was trapped in the flask, the rubber tubes leading to the flask were clamped with a hæmostat and then detached from the cannulæ The flask was then shaken for 1 to 5 minutes and re-attached Circulation of the blood caused vaso-dilatation which varied directly in degree with the amount of shaking Circulation of blood in the flasks that had been detached for the same length of time and re-attached without shaking caused either very slight or no vaso dilatation This experiment proved that the vaso-dilator property may be acquired by traumatism without exposure of the blood to air This was further demonstrated when the blood was withdrawn from and returned to the flask under paraffin If shaken while outside, it caused vaso-dilatation when circulated but it had little or no dilator effect if it had not been shaken

In order to test for vaso-dilator action of untraumatised venous blood collected from the limb during the period of reactive hyperæmia following the release of arterial obstruction, a cannular system with flask was inserted in the femoral vein as well as one in the femoral artery With the circulation on the venous side passing through the flask, the artery was obstructed, and then released after 2 to 3 minutes The venous flask was short-circuited 45 to 60 seconds later, clamped off and transferred to the arterial side Its blood which had come from the limb during the height of the period of reactive hyperæmia was then circulated, and in numerous trials in four experiments it produced either no vaso-dilatation as shown in Fig 4 or a very faint vaso-dilatation such as might result from contact of the stagnant blood with the flask during the time in which the test was being carried out Blood which is stagnant in the flask for more than 5 minutes may acquire slight vaso-dilator properties which increase with prolongation of stagnation The effect of shaking should be regarded as merely an acceleration of the process

Light was excluded by the use of a hood and of flasks that were coated with black paint Blood that was traumatised and circulated with the light excluded caused vaso-dilatation while untraumatised blood did not The temperature of the blood was determined by the

use of a flask with a bung in the top which held a thermometer A single-jacket flask holding 40 c c of blood was detached after clamping the

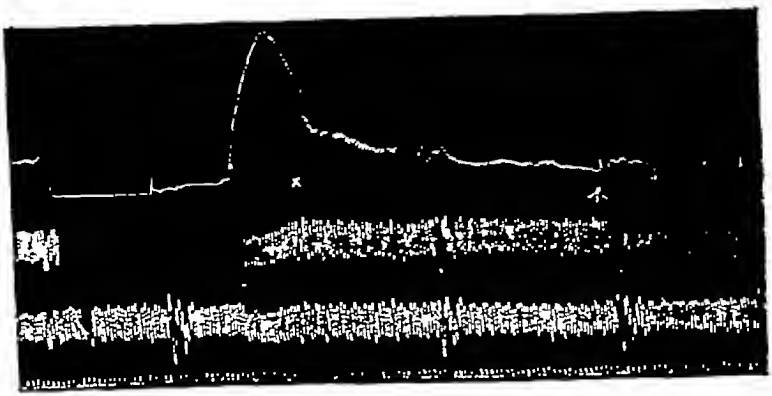

Fig 4. Blood, short-circuited in venous flask at ×, 1 minute after release of 3 minutes' obstruction of femoral artery and, transferred to the arterial side, caused practically no vaso dilatation when circulated at the arrow point

rubber tubes, heated or cooled, re-attached and circulated without shaking Variations in temperature of the blood within 2° to 30° C had no effect unless the blood was kept out of circulation for several minutes Traumatised blood, the temperature of which was kept constant, always gave vaso-dilatation

The hydrogen-ion concentration was determined before and after shaking for 4 to 5 minutes and was found to be either unaltered or very slightly shifted toward the alkaline side In order to exclude heparin as a possible factor the blood of the dog was defibrinated by withdrawing, whipping, and re-injecting 250 c c at a time for 10 or 12 times The cannulæ were then inserted into the artery without the use of an anti-coagulant In three attempts the blood coagulated in the tubes before the experiment could be completed In one successful experiment the circulation continued through the tube for 20 minutes and both arterial and venous bloods were found to produce vaso-dilatation when traumatised before circulation. 0 7 grm. of hidrudin was used in two experiments and the results were the same as those obtained with heparin Consequently heparin cannot be regarded as a factor in the causation of the vaso-dilatation. Heparinised blood that had not been shaken was centrifugated and the plasma drawn off When plasma alone was circulated in the limb, it always produced vaso-dilatation This action may be due to the traumatism during centri-

fugation, or to diminished viscosity But on the other hand, circulation of the remaining unwashed corpuscles also produced after a temporary decrease in volume a dilatation of the limb The limb constriction was interpreted as being due to the high viscosity and the dilatation which followed to the vaso-dilator property acquired as a result of the traumatism

From these experiments it was concluded that blood when traumatised as by shaking for 10 seconds to a few minutes acquires a vaso-dilator property which is independent of exposure to light or air and of changes of temperature and of changes in O_2 or CO_2 content and of hydrogen-ion concentration All of the reactions described above were also observed in seven experiments in which the sciatic and crural nerves were not cut

Two theories were entertained as to the nature of the change produced by traumatism of the blood which gives it the vaso-dilator property One was that a vaso-constrictor substance is destroyed and the other that a vaso-dilator substance is formed or liberated either in the blood or by the tissues when in contact with traumatised blood The pituitary principle and adrenaline are the two vaso-constrictor substances that have often been held responsible for the maintenance of normal vascular tone The evidence in favour of either as the important factor is both meagre and fragmentary Krogh(4) believed, mainly as a result of chemical studies and of perfusion of the vessels of a frog with a dialysate of ox blood, that pituitrin in a concentration of around 1 to 100,000 is the active substance Dale and Richards(5) regarded it as possibly adrenaline

From a chemical standpoint both adrenaline and the pituitary principle are far too stable in blood to be destroyed during the few seconds of shaking which are necessary to give it vaso-dilator properties If, however, this be the case, then it is difficult to imagine that these substances would remain unchanged for several minutes in untraumatised shed blood Attempts were made to neutralise the vaso dilator action of traumatised blood by the addition of minute quantities of adrenaline or pituitary extract (B D H) and to find whether the amounts necessary for such neutralisation are of the order which would be expected to be present in the normal circulating blood When adrenaline was added to 30 c c of untraumatised blood in a concentration of 1 to 500 million it was found on circulation that slight vaso-constriction occurred lasting 20 to 40 seconds 1 to 100 million produced a conspicuous decrease in limb volume and increase in limb pressure

But when shaken blood was treated with 1 to 500 million of adrenaline the usual vaso-dilatation followed on circulation as if nothing had been added. Even when the adrenaline concentration in the shaken blood was raised to 1 to 200 and 1 to 100 million it still caused the usual amount of increase in limb volume and decrease in limb pressure, but the recovery was more rapid than normal and slight constriction followed. It was found in some experiments that circulation of shaken blood plus as much as 1 in 20 million of adrenaline would at first produce slight increase in limb volume and fall in limb pressure which were followed by rapid and marked decrease in limb volume and slight increase in limb pressure Unshaken blood to which adrenaline was added in the above concentrations produced in our experiments vaso-constriction only Similar experiments were made with pituitary extract (B D H) and it was found that as much as ten times the amount necessary to give a slight constrictor effect to 30 c c of untraumatised blood would not prevent beginning vaso-dilatation when added to well-shaken blood, although with large concentrations of pituitary extract the vaso-dilatation was quickly cut short and followed by vaso-constriction (Fig 5)

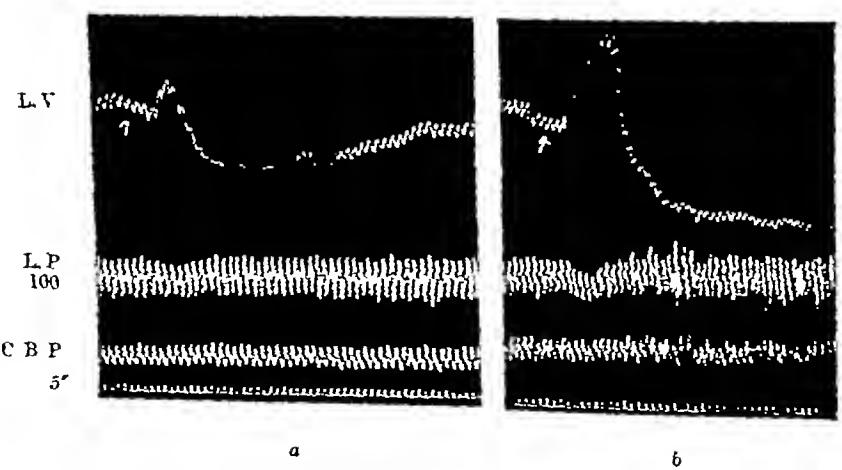

Fig 5, Exp. 15 a Shaken arterial blood plus 1 to 100 million adrenaline b Shaken arterial blood plus 1 to 1200 B.D H pituitary extract

Still larger amounts of both adrenaline and pituitary extract completely prevented the vaso-dilator action of shaken blood and caused vaso-constriction from the beginning It became apparent that the concentrations of adrenaline and pituitary extract which are necessary to

prevent the vaso-dilator effect created by traumatism are much higher than those to be expected in the arterial blood, consequently it is highly improbable that the vaso-dilatation is the result of the destruction of these substances

The theory that the vaso-dilator action of traumatised blood is due to the formation or liberation of a vaso-dilator substance would seem the more plausible one Injury to the red blood cells with consequent hæmolysis and liberation of vaso-dilator substances of a histamine-like nature was considered In order to test this theory experiments were performed with different amounts of (*a*) blood traumatised in varying degrees, (*b*) laked blood, and (*c*) blood to which different amounts of histamine were added

(A) *Traumatised blood* The amount of traumatism was increased by shaking for increasing lengths of time either with or without glass beads The quantity of blood circulated was varied by the use of flasks holding 30, 75, 220 and 475 c c respectively When a 30 to 75 c c flask was used no extra blood was employed, the circulation being started through the flask filled with physiological salt solution When a 220 or 475 c c flask was used it was filled at the start with heparinised blood from another dog and the circulation was allowed to pass through the flask until the two bloods were thoroughly mixed No difference was noted in the effects of blood whether it was that of donor or recipient or a mixture of the two

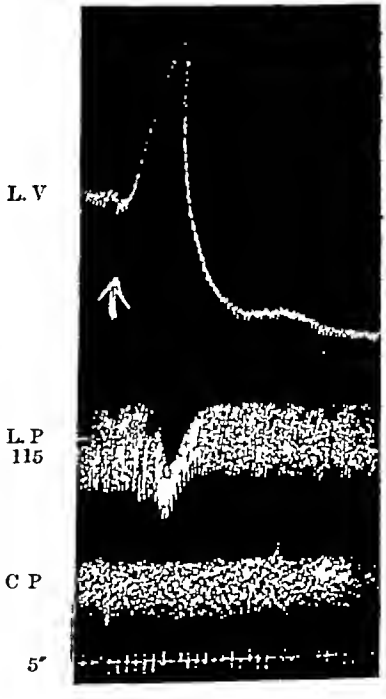

Fig 6 Circulation of arterial blood shaken 20 minutes with bead.

When 30 c c of blood were used, it was found that a maximum dilator effect was obtained by shaking with a bead for approximately 2 minutes, and again as with blood shaken without a bead, there was no change in the degree of vaso-dilatation when the blood was shaken for 8 to 10 minutes But shaking with a bead for 20 minutes produced

moderate vaso-dilatation followed quickly by limb constriction lasting for 3 or 4 minutes (Fig 6) There is much froth formed by prolonged shaking so that 40 to 50 c c of blood were used in order to insure enough to fill the 30 c c flask When 30 c c that had been shaken with a bead for 55 minutes were circulated immediate limb constriction and slight rise in limb pressure followed Five minutes were required for the limb to return to its former volume, after which there was still further increase in volume and diminution in volume pulsations (Fig 7)

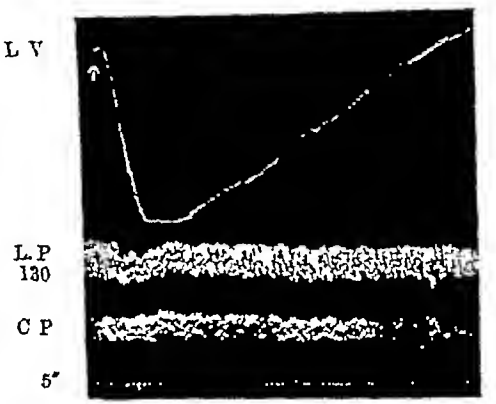

Fig 7 Circulation of 30 c.c blood shaken with beads for 55 minutes

Increase in amount of blood circulated A surprising finding was that when more than 30 c c of blood shaken for 2 to 5 minutes was circulated the vaso-dilatation did not increase proportionately with increase in the amount of blood 75 c c produced slightly more effect than did 30 c c but 220 c c or 475 c c produced no greater nor more prolonged vaso-dilatation than did 75 c c Approximately 4 minutes were required for 475 c c to pass out of the flask, when that amount was circulated, vaso-dilatation reached its maximum in 25 to 35 seconds, and wore off in 60 to 90 seconds, limb volume and limb pressure then remained constant despite the fact that shaken blood circulated for about $2\frac{1}{2}$ minutes more (Fig 8) The vessels apparently acquire the property of resisting the vaso dilator effect of such traumatised blood after approximately 30 seconds, and quickly assume their former contractile state, despite the fact that the same kind of blood still circulates in them 475 c c of blood, shaken with beads in a Florence flask for 10 minutes caused vaso dilatation about equal to that produced by blood shaken for 2 minutes, and no vaso constriction followed But

475 c c of blood that had been shaken with beads in 50 c c lots for 1 hour, so that the amount of traumatism was extreme, produced immediate diminution in limb volume and slight rise in limb pressure, similar

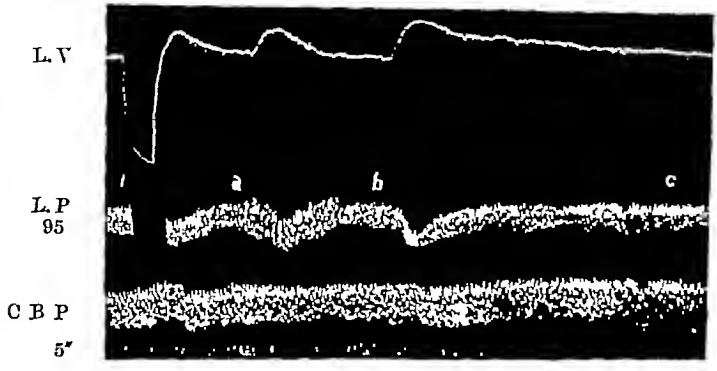

Fig 8, Exp 54. 1 Reactive hyperæmia *a* 30 c c arterial blood shaken 2 minutes *b* Circulated 475 c c arterial blood shaken with beads for 5 minutes *c* Approximate time when traumatised blood all out of flask

to the effect of 30 c c shaken for 1 hour The limb constriction lasted for 6 minutes after which it wore off and limb dilatation followed while limb pressure returned to normal General blood-pressure remained unchanged throughout the experiment

It was thus apparent that blood which had been slightly or moderately traumatised produced vaso-dilatation on circulation through the limb, but increase in the amount of blood circulated beyond 75 c c did not increase the amount or duration of the vaso-dilatation The circulation of blood that was severely traumatised produced first vaso-dilatation followed by limb constriction and rise in limb pressure, and blood that was still more severely traumatised produced limb constriction and rise in limb pressure from the onset Increase in the amount of severely traumatised blood circulated caused prolongation of the period of limb constriction

To test for hæmolysis, equal quantities of blood that had been similarly shaken for varying lengths of time were centrifuged for 30 minutes and their plasma examined for the presence of hæmoglobin The plasma of 50 c c of blood shaken by hand in a 100 c c flask up to 1 minute usually showed no hæmoglobin visible to the naked eye, but shaking for 2 to 3 minutes usually produced a definite reddish tint, and the greater the traumatism, the more intense became the

discoloration with hæmoglobin. Shaking for 1 hour produced very marked discoloration. The amount of hæmoglobin in each specimen of plasma was determined colorimetrically by comparison with whole blood, the hæmoglobin content of which was obtained by means of the Sahli hæmoglobinometer In one experiment where whole blood contained 17 87 grm. of hæmoglobin per 100 c c the plasma of unshaken blood centrifuged for 30 minutes contained 0 0469 grm. per 100 c c , that of blood shaken for 30 seconds 0 0573 grm. per 100 c c , that of blood shaken for 1 minute 0 0677 grm. per 100 c c , and that of blood shaken for 2 minutes 0 0791 grm. per 100 c c When blood was shaken with a bead for 12 minutes, the plasma contained 0 908 grm. per 100 c c , when shaken for 20 minutes, it contained 1 418 grm. per 100 c c , and when shaken for 55 minutes, it contained 3 689 grm. per 100 c c Thus unshaken blood showed after centrifuging only a faint hæmolysis, while after shaking for 55 minutes more than one-fifth of the hæmoglobin was liberated.

(B) *Laked blood* In 16 experiments blood was laked by freezing and thawing Freezing was accomplished most conveniently by immersion of the blood contained in Pyrex tubes in liquid oxygen. Estimations with the Sahli hæmoglobinometer showed about 90 p c of the hæmoglobin in the plasma Amounts of the hæmolysed blood varying from 0 07 c c to 220 c c were circulated and untraumatised blood was diluted with different amounts of hæmolysed blood before circulation.

Injection of 0 07 c c of laked blood into the tube distal to the flask always produced a small amount of vaso-dilatation. The amount of vaso-dilatation increased with increase in the amount injected up to 0 5 or 0 7 c c , dependent on the experiment and on the degree of hæmo-

Fig 9 Exp 80 *a* Circulation of 30 c c of arterial blood shaken 2 minutes *b* 0-07 c c of 0-005 p c histamine *c* 0-07 c c of hæmolysed blood. *d* 0 15 c c of hæmolysed blood *e* 0-7 c c of hæmolysed blood *f* 1 c c of hæmolysed blood.

of it When, in an experiment on a dog weighing 10 5 kgm , 10 mgm of histamine were added to 475 c c of blood, there resulted on circulation, a rapid increase in limb volume and fall in limb pressure followed by fall in general pressure (Fig 11) But within 75 seconds these effects

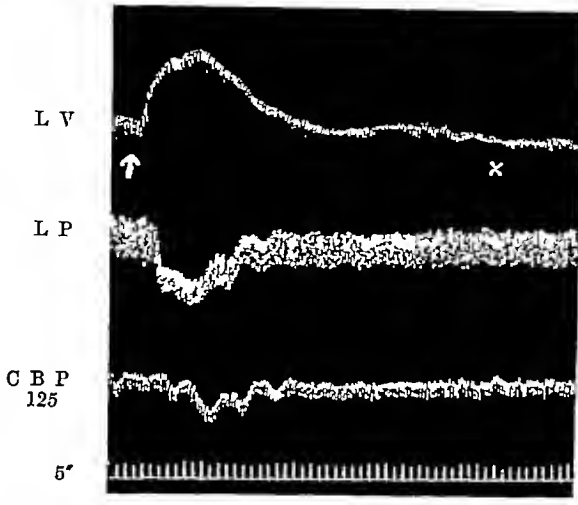

Fig 11, Exp 64 10·5 kgm dog Circulated 475 c c blood withdrawn and mixed with 10 mgm histamine Flask empty at ×

had largely worn off despite the fact that blood containing the same proportion of histamine continued to enter the limb and from there the general circulation for at least 2½ minutes longer On the other hand, when 30 c c of blood containing 10 mgm of histamine were circulated, the blood-pressure in both femoral and carotid arteries fell rapidly to 15 mm of mercury and the animal showed evidences of extreme and prolonged shock from which it had only partially recovered at the end of an hour

Histamine was added in varying amounts to 30 c c of hæmolysed blood It was found on circulation to counteract the constrictor effects and to produce vaso-dilator effects similar to but somewhat less marked than those produced when it was added to non-hæmolysed blood

When the flask was connected with the femoral vein, the circulation of 475 c c of venous blood containing 2 mgm of histamine caused a fall in general blood-pressure of 40 mm Hg which was gradually recovered from in 10 minutes

There was no instance of the reversed action of histamine as found by Dale and Richards when it was added to artificial perfusion fluids

In four experiments 30 c c of blood were withdrawn and defibrinated before heparin was injected. On subsequent circulation, it was found to produce vaso-dilatation similar to that produced by shaken blood. Whipping for defibrination was kept up for 5 to 6 minutes In two experiments, between 500 and 600 c c of blood from another dog were defibrinated by whipping for 15 to 20 minutes with a wire wisp Circulation first of 30 c c and subsequently of 475 c c of this defibrinated blood produced vaso-constriction similar to that of larger quantities of hæmolysed bloods The hæmoglobin content of the serum was not determined in any of the experiments with defibrinated blood but it was undoubtedly higher in the second set than in the first

A comparison of the results obtained by circulation of varying quantities and intensities of traumatised and hæmolysed bloods reveals very great similarity of action Blood traumatised to a slight or medium extent produces vaso-dilatation, when traumatised more extensively it produces vaso-dilatation followed by vaso-constriction and when traumatised still more extensively it produces vaso-constriction only Hæmolysed blood when injected into the tube in very small quantities or mixed with the unshaken blood of the 30 c c flask in somewhat larger quantities causes vaso-dilatation, when these quantities are moderately increased they cause vaso-dilatation followed by vaso-constriction and when they are markedly increased they cause vaso-constriction only

Increase in the quantity of slightly or moderately shaken blood up to approximately 75 c c increases the intensity and duration of vaso-dilatation, but quantities greater than this produce no increase in the intensity or duration of the vaso-dilatation. The same is true of unshaken blood diluted with very small quantities of hæmolysed blood Increase in the quantity of blood that has been traumatised to a point where it produces only vaso-constriction on circulation, causes prolongation of the period of vaso-constriction. This is also true of hæmolysed blood when circulated in moderate or high concentrations

When the effects of traumatised and hæmolysed bloods are compared with those of blood treated with histamine, there are some points of similarity, and others of marked difference There is a similarity of action in that circulation of 30 c c of untraumatised blood containing a very small amount of histamine causes vaso-dilatation in the limb without alteration of general blood-pressure, also further increase in the quantity of blood circulated containing histamine in this amount

does not proportionately prolong the period of vaso-dilatation There is a marked difference in the action in that increase in the amount of histamine in a given quantity of blood beyond that which produces only dilatation in the limb, causes further limb dilatation and also dilatation of systemic capillaries with fall in general blood-pressure The fall in general blood-pressure is also proportionately greater when the amount of histamine is increased, large amounts causing histamine shock

The cause of the vaso-dilatation in the limb which results when mildly traumatised blood or a minute quantity of hæmolysed blood is circulated is apparently the same in either case and is a substance liberated from the broken down red blood cells

The facts that increase in the traumatism or laking beyond a slight degree does not increase the vaso-dilator action, that marked increase causes vaso-constriction, and that the addition of increasingly greater amounts of histamine to blood whether untraumatised, traumatised, or hæmolysed always produces correspondingly greater vaso-dilatation, speak against histamine as the cause of the vaso-dilatation

The cause of the vaso-constriction produced by the circulation of extremely traumatised or extensively hæmolysed blood may also be regarded as a substance liberated from the red blood cells, but active as a vaso-constrictor only when present in relatively high concentration

The question of whether the vaso-dilatation produced by a small traumatism of blood and the vaso-constriction produced by an extensive traumatism are due to the liberation of different amounts of one and the same substance or of two different substances must at present be left open

Summary and Conclusions

1 The use of a vivi-perfusion apparatus devised by Dr G V Anrep is described

2 Arterial or venous blood rendered incoagulable by heparin or hirudin acquires vaso-dilator properties when outside the body

3 Traumatism of the blood up to a certain point hastens the development of vaso-dilator properties

4 The vaso-dilator properties of such blood are independent of changes in O_2, CO_2, H-ion concentration, temperature, and exposure to air or light They appear not to be the result of destruction of adrenaline or of pituitary principle while the blood is outside

5 The plasma of such blood shows evidences of a slight amount of hæmolysis

6 Laked blood, when injected in small quantities or circulated in high dilution with untraumatised blood also produces vaso-dilatation

7 Blood that has been severely traumatised acquires vaso-constrictor properties Its plasma shows a moderate or high degree of hæmolysis

8 Moderate or high concentrations of laked blood also produce vaso-constriction when circulated in larger quantities

9 These vaso-motor changes are apparently due to the action of the hæmolysed blood which when present in the circulated blood in small amounts causes vaso-dilatation and in large amounts causes vaso-constriction.

10 The vaso-dilator properties of traumatised blood are as far as the evidence shows not due to the liberation of histamine

11 Untraumatised blood collected from the femoral vein during the period of reactive hyperæmia following arterial obstruction possesses no special vaso-dilator properties

12 Blood vessels when perfused with large quantities of blood possessing vaso-dilator properties, whether due to traumatism or adding hæmolysed blood or histamine dilate at the onset but rapidly acquire the power of resisting the vaso-dilator action.

Thanks are due to Dr Anrep for assistance throughout the course of the work, and Prof Lovatt Evans for suggestions in connection with the work on hæmolysed blood.

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THE NERVOUS MOTIVE ENERGY

REPLY TO SYBIL COOPER AND E D ADRIAN

By I ATHANASIU (*University of Bucarest*)

Since Piper's(1) demonstration, by means of the string galvanometer, that the action current of the muscles in voluntary contraction shows a great number of oscillations during the unit of time, the question arose whether the rhythm of these oscillations was derived from the muscles or the nervous system

The controversy which arose over this question between Garten(2) who attributed it to the muscle, and Piper(3) who attributed it to the nervous system, is already known

Forbes and Rappleye(4), in trying to elucidate the problem by experiments made on man, warmed and cooled the muscle of the fore-arm to which were applied non-polarisable electrodes They found that the frequency of the oscillation in the electromyogram rises with the warming and diminishes with the cooling of the muscle, which should prove that their rhythm depended on the muscle and not on the nervous system The latter is supposed to send to the muscle, according to these authors, 300–1000 vibrations per second and even more But they do not bring any experimental proofs to back their views

Cooper and Adrian(5) have taken up the question and made experiments on frogs with brains destroyed Their researches aimed at distinguishing the share of the muscle from that of the nervous system in the rhythm of the oscillations in the reflex electromyogram and to that end they separately and alternately cooled the muscle and the spinal cord When the muscle was cooled, the cord was kept by certain methods at a constant temperature, and vice versa The non-polarisable electrodes were placed on the gastrocnemius muscle and a fore-limb was stimulated either by mechanical or electrical means (induction shocks) The photographic registering apparatus moved at a speed of 15 cm per sec The authors do not mention the physical constants of the string of the galvanometer

Cooper and Adrian have counted, in the reflex electromyogram thus obtained, only the large oscillations on one side and on the other the

total number They found that cooling the muscle (the temperature of the spinal cord remaining constant) causes little change in the rhythm of oscillation in the reflex electromyogram, but that cooling the cord (the temperature of the muscle remaining constant) changes it more

Thus taking the average of the number given by these authors, we find

(*A*) *Cooling the muscle*

Temperature	Total number of oscillations per sec.
11°–11 5°	118
20°	122
22°–25°	128

(*B*) *Cooling the cord*

5°	95
6°	105
7°	118
14°	126
20°	145

From these experiments Cooper and Adrian concluded that the rhythm of the oscillations in the reflex electromyogram follows exactly the frequency of nervous discharge of the spinal cord, which would be about 120–150 per sec in the frog at room temperature Consequently the rhythm depends on the nervous system and not on the muscle It is supposed that the number of nervous vibrations is not superior to the frequency of the muscle response The authors state at the same time that when warming the cord the large oscillations become more numerous and their intervals more regular In order to explain this phenomenon, they suppose that the amplitude of these oscillations depends on the number of muscle fibres contracted at the time and that this number would be higher when the cord is warmed, because of a larger number of the motor neurones coming into action for the same movement

This summarises as faithfully as possible the results reached by Cooper and Adrian

Commenting on these results, they find them in contradiction with those I have obtained in my own work on the nervous motive energy (6)

We shall separately examine each question discussed by these authors

1 *The existence of electro-neuro-motive oscillations in the voluntary and reflex electromyogram* My researches allowed me to distinguish in every electromyogram, voluntary or reflex, the presence of two kinds of oscillations some of variable amplitude (large and middle-sized) which

I have called *electro-muscular* and the others of very small but regular amplitude, appearing in groups of 2–5, called *electro-neuro-motive*

In order to be able properly to distinguish these two kinds of oscillations, we may take the following records Figs 1 and 2, Plate I, showing voluntary electromyograms of the flexor muscles of human fingers and Figs 4 and 5, Plate II, showing reflex electromyograms of the gastrocnemius of the frog The groups of small oscillations included between the white dotted lines are those which I call electro-neuro-motive, all the others are the electro-muscular ones The rhythm of the first ones is marked above each group I will briefly point out the reasons which lead me to consider that those small oscillations represent the action current which accompanies the nervous motive energy

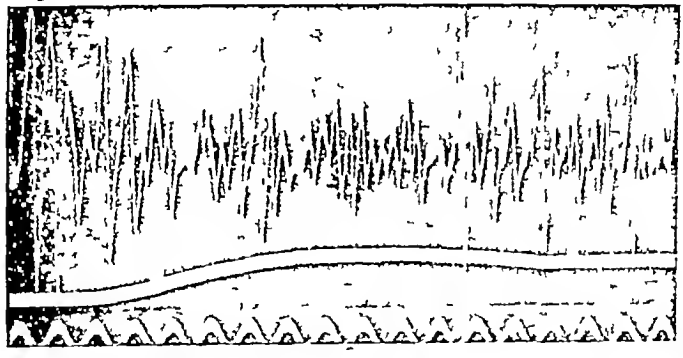

Fig 1

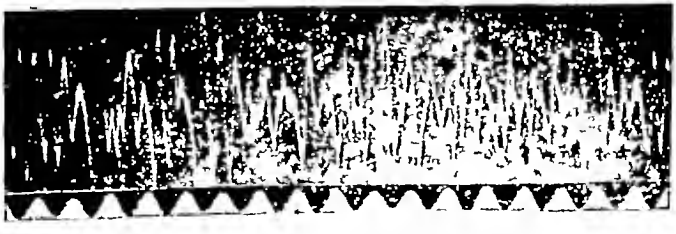

Fig 2

Fig 1 E = Direct electromyogram of a neuromuscular preparation. Number of electric stimuli applied to the nerve = 982 M = Myogram T = Time in one hundredths of a second. String of silvered glass Diameter = 2 5μ. Resistance = 6500 ohms Tension = 1 5 mm per 0 001 v

Fig 2 Reflex electromyogram of frog without brain. Exterior temperature = 24° Gastrocnemius muscle String of silvered glass Diameter = 2μ. Resistance = 9000 ohms. Tension = 0 8 mm per 0 001 v An amplifier in the circuit. Mechanical stimulation of the fore limb T = Time in one hundredths of a second.

When we register the action current of a muscle-nerve preparation, after the nerve has been stimulated, one can see that up to 300 stimuli per sec the oscillations of the galvanometer follow the frequency of the stimulus very exactly, though their amplitude lessens on account of the fatigue Above 300 stimuli per sec the form of the electromyogram becomes more and more irregular and approaches the reflex or voluntary electromyogram. Fig 1 is a direct electromyogram and Fig 2 a reflex electromyogram of the frog

The likeness between the two is perfect, the same large, middle-sized and small oscillations are in the two The small oscillations, of regular amplitude, which are in the direct electromyogram, follow between certain limits the rhythm of the stimulus What other conclusion can be drawn from this likeness, unless that the muscle in voluntary or reflex contraction also receives a number of stimulations greater than 300 per sec ? And if we consider the groups of quite small oscillations as representing the nervous stimulus, we find in fact that their frequency is included between 300 and 550 oscillations per sec , in the hot summer time we have even found 600 per sec

The very irregular form of the other oscillations (large and middle-sized) which represent the action currents of the muscular contraction, may be explained by the intervention of the refractory period of the muscle, which puts a limit to the frequency of its responses The following diagram (Fig 3) shows the mechanism by which this refractory period has such an effect on the form of the electromyogram (voluntary or direct)

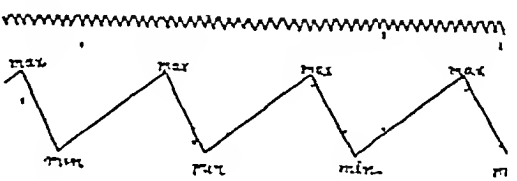

Fig 3

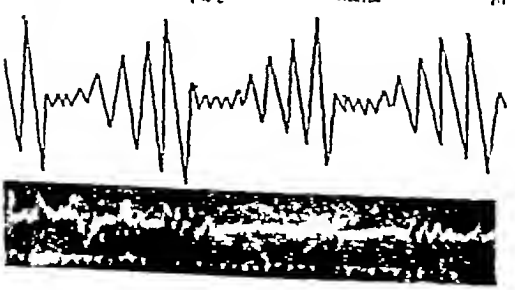

Fig 4.

We suppose a rhythmic stimulus giving a certain number of interruptions in the unit of time The muscle having phases of maximum and minimum excitability, the intensity of its responses—expressed by the amplitude of the electromuscular oscillations—will be strongest during the maximum phase and weak during the minimum phase It is even possible that the muscle may not respond at all during this last period, and if so the galvanometer will record only the action current which accompanies the nervous motive energy This action current is expressed by the small oscillations of a regular amplitude, which are to be seen as well in the direct electromyogram as in the voluntary and reflex one

One may get reflex electromyograms from cooled frogs the forms of which considerably resemble our diagram, as may be seen in Fig 4 The large oscillations are sufficiently regular There are among them middle-sized and groups of quite small ones Our explanation therefore finds a very important support in this electromyogram Again Cooper and Adrian show in Fig 6 of their paper an electromyogram of the frog with the cord cooled to 4 5°, which bears a very close likeness to our diagram

We can conclude from the preceding observations that the oscillations of every electromyogram, reflex or voluntary, have two different origins (*a*) the large and middle-sized are muscular waves as far as the energy is concerned, and show the action current which accompanies the muscle twitch, (*b*) the very small ones are of nervous origin, and represent the action current which accompanies the nervous motive energy

The frequency of the first is generally between 80 and 150 per sec in the mammalia and frog, reaching even 200 in the latter during the great summer heat The frequency of the second is much higher, it comprises 300–500 oscillations per sec in the mammalia and 300–600 in the frog during summer time One sees, then, how irrational it is to add these two kinds of oscillations together in the voluntary or reflex electromyogram

In order to obtain further evidence as to the nervous origin of the small oscillations in those electromyograms we recorded the action current of the nervous motor system (brain + cord or brain + sciatic nerve) Fig 3, Plate I, shows an example of electroneurogram of the guinea-pig (brain + sciatic nerve) The two large oscillations belong to the heart, they are electrocardiograms The numerous small oscillations are sufficiently alike to those of the voluntary electromyogram as to their form and number

But Cooper and Adrian mention in their memoir "Our own records

of the normal electromyogram (of the frog, the cat and man) show occasional clusters of very small waves of high frequency, but we have never been able to make out any clear separation of two such types of waves as Athanasiu describes, since there are always many of intermediate size It should be added, however, that we have not made any detailed statistical examination of our records" These statistics have been made by me upon hundreds of records and I consider them as accurate

If these authors did not succeed in clearly separating the two kinds of oscillations described above, the explanation of their failure is quite simple It consists in the slow speed of their registering apparatus, which did not exceed 150 mm. per sec , whereas I insist in my work upon the fact that 500 mm. per sec is necessary at least

The number of electro-neuro-motive oscillations being of 300–600 per sec in the frog in summer, it follows that one millimetre of the record of these authors must contain 2–4 of the small oscillations, a condition materially impossible, because the shadow of the string itself only measures 3–4 tenths of a millimetre The frequent black spots seen on those records are due to nothing else than the electro-neuro-motive oscillations heaped together

Moreover, we do not find in the work of these authors the physical constants of the string used and especially its tension. I have demonstrated already that a string under slight tension cannot follow the rhythm of rapid oscillations, and if such has been the case with Cooper and Adrian's experiments it is still easier understood why they have not been able to observe the electro-neuro-motive oscillations properly My researches have shown that Piper's fear of tightening the string, on account of its own vibrations which would interfere with the record, was by no means justified On the contrary, the tight string follows with the greatest fidelity the swiftest variations of the current, without any interference, as I have convinced myself by repeatedly testing the string galvanometer

Therefore Cooper and Adrian's objection, on the existence and the meaning of the electro-neuro-motive oscillations, is by no means justified

(2) *The large and middle-sized oscillations represent the action current of the muscle twitch* In my researches on the action current of the muscle in voluntary contraction, I showed that the large and middle-sized oscillations of the electromyogram are the expression of the action current of the muscle twitch The following additional proofs may be brought in support of this

(*a*) When we record the action current of a muscle-nerve preparation (gastrocnemius muscle + sciatic nerve) successively in isotonic and isometric contraction, we find that the amplitude of the oscillations is considerably diminished in the second case, because the muscle is unable to contract and gives a very faint twitch In Figs 5 and 6 this is shown very clearly

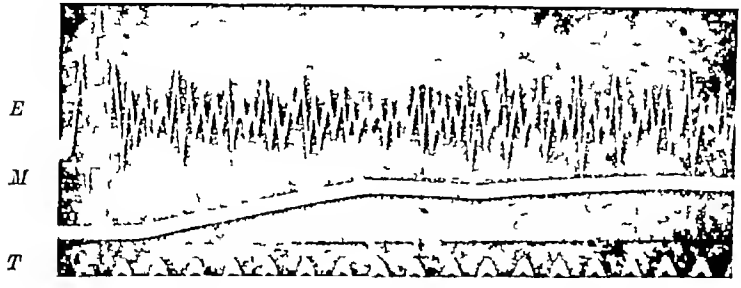

Fig 5

Fig 6

Fig 5 E = Direct electromyogram of a neuromuscular preparation. Frequency of stimulation applied to the nerve = 569 per sec. Isotonic contraction. M = Myogram String of silvered glass Diameter = 2 5μ. Resistance = 6500 ohms Tension = 1 5 mm per 0 001 v Time in one hundredths of a second.

Fig 6 E = Electromyogram of the same preparation in isometric contraction M = Myogram Same string and same tension

(*b*) By the help of a special apparatus, described in my first work (p 11) I have, in collaboration with Bull, recorded the longitudinal vibrations of the flexor muscle of the fingers and their action current, with two different string galvanometers, as it is shown in Fig 7

We obtained the records I and II The first one (I) is the voluntary electromyogram of the flexor muscles of the fingers, II represents the longitudinal vibrations of the same muscles (muscle twitch), converted into electric vibrations by the apparatus F held in the hand If the

contraction of the muscle were perfectly continuous the record II would be a straight line, because no current could have been produced in the

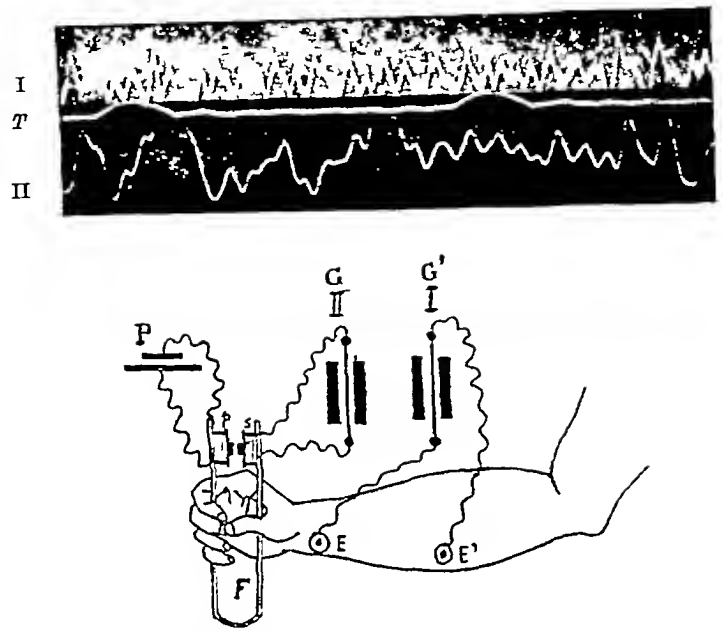

Fig 7 I = Voluntary electromyogram of the flexor muscles of the fingers given by the galvanometer G' T = Time in one fifth of a second. II = Electric record of the longitudinal vibrations of the same muscle, given by the galvanometer G and the apparatus F held in the hand. The first coil receives the current from the battery P The secondary coil (s) is in the circuit of the galvanometer G E and E = Non-polarisable electrodes I = String of silvered glass Diameter = 3 5μ. Resistance = 6500 ohms Tension = 1 5 mm. per 0·001 v II = String of silvered glass Diameter = 3μ. Resistance = 7000 ohms. Same tension as I string

secondary coil (s) But we see that this record has on the contrary a great number of oscillations, which almost copy the form of the large and middle sized waves of the electromyogram Consequently both have the same origin and represent the twitches of the muscle when in continuous contraction (tetanus) That is the reason why I have called the large and middle-sized oscillations of the voluntary or reflex electromyogram electromuscular oscillations They are of muscular origin, as far as the energy is concerned, because they represent the action current which accompanies the muscle twitches But as to their frequency and amplitude they depend directly on the nervous system, just as the oscillations of the direct electromyogram depend on the stimulus applied to the

nerve Cooper and Adrian are therefore unjustly attributing to us the opinion, similar to Garten's, that the rhythm of these oscillations is due to a peculiar quality of the muscle

If these authors had been able to record distinctly the two kinds of oscillations (the electromuscular and electro-neuro-motive ones) they would have seen that cooling the cord diminishes the frequency of both of them, as we have shown by repeating the experiments of Cooper and Adrian We give in the following table the average number of the electromuscular and electro-neuro-motive oscillations in the frog, before and after cooling either the spinal cord only or the whole frog

(*A*) *Cooling the spinal cord*

	Temperature in the œsophagus	Number of electro muscular oscillations	Number of electro neuro motive oscillations	Relation Elect. neur mot. oscil Elect musc. oscil.
Before cooling	23 – 25°	170	545	3 2
„ „	23 – 25°	201	583	2 9
After cooling	7 8–10°	130	440	3 4
„ „	7 8–10°	135	412	3 0

(*B*) *Total cooling*

Before cooling	21 – 24°	192	500	2 6
„ „	21 – 24°	176	516	2 9
After cooling	7 – 10·5°	165	430	2 7
„ „	7 – 10 5°	138	455	3 3

It follows from the above table that cooling only the cord diminishes the frequency of the electromuscular oscillations and therefore the frequency of the muscle twitches too, as has been demonstrated by Cooper and Adrian But it diminishes also the number of electro-neuro-motive oscillations, therefore, the number of stimulations sent by the nerve centres to the muscle The rhythm of the former is consequently strictly dependent on the rhythm of the latter, as I have affirmed, and all that affects the nervous centres necessarily influences the two sorts of oscillations Such is the effect of poisoning these centres by alcohol(7), which—like cooling—diminishes the number of the neuro-motive vibrations, from which there results a diminution in the number of muscle twitches

Figs 3 and 4 (Plate II) are examples of reflex electromyograms of gastrocnemii muscles of the frog cooled either entirely or the spinal cord only

(3) *The frequency of the nervous-motive vibrations is superior to the number of responses that the muscle can give* We quote Cooper and Adrian's memoir "Cooling the cord should reduce the frequency of the

impulses in the nerve, but if this is still too rapid for the muscle to follow, the effect would be either to leave the muscular response unchanged or else to make its frequency increase" Our studies of the direct electromyogram showed us that its form becomes similar to that of the voluntary electromyogram as soon as the frequency of stimulation applied to the nerve surpasses the frequency of response which the muscle can follow Now the mere irregularity of the voluntary electromyogram suffices to indicate that the number of nervous impulses must surpass the limit of the muscle response, because, if the number were within these limits, as Cooper and Adrian suppose, the amplitude of the oscillations of the voluntary electromyogram would be regular, as it is in the direct electromyogram with stimulation not above 300 per sec Fig 8 shows, in fact, the form of a direct electromyogram with the nerve stimulated with 157 excitations per sec

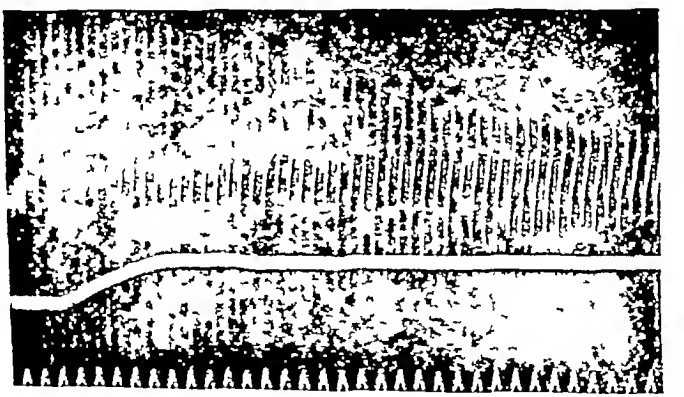

Fig 8 E = Direct electromyogram of gastrocnemius muscle of the frog Number of stimulations applied to the nerve = 157 per sec. M = Myogram. T = Time in one hundredths of a second. String of silvered glass Diameter = 2 5μ. Resistance = 6500 ohms. Tension = 1 5 mm. per 0·001 v

Such would be the form of the reflex electromyogram if the opinion of Cooper and Adrian were correct, namely "that the maximum rate of discharge from the cord in the frog at room temperature is 120–150 per sec , and that the frequency of the oscillations in the electromyogram is usually identical with the frequency of discharge from the cord" Now, on the contrary, the form is very irregular, but this does not happen by chance There is a close enough relation between the number of nervous motive vibrations and that of the muscular responses included in every voluntary or reflex contraction In fact I have found in records of

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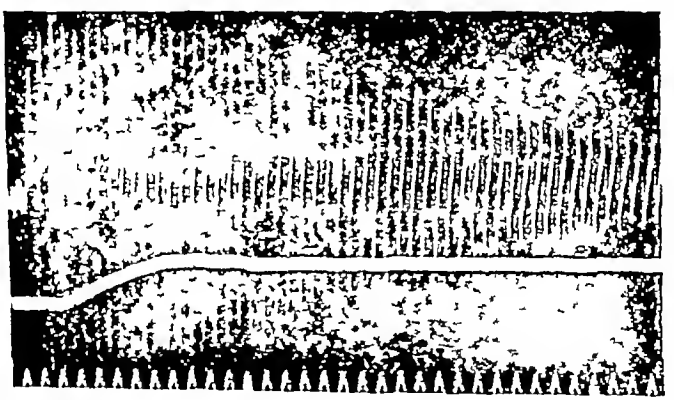

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voluntary electromyograms made in a series of animals(8) that this relation is almost a constant quantity The relation oscillates around the number 4, whether it is caused by a very quick movement, as the flight of a bird, or a very slow one, as the progress of the snail Thus the average is four nervous vibrations for one muscle response (twitch), and if the nervous centres are cooled or poisoned by alcohol and thus diminish the frequency of the vibrations sent to the muscle, one distinguishes at the same time a proportional reduction in the frequency of the muscle response notwithstanding the fact that the muscle still receives a number of nervous stimulations superior to its rate of response These experiments prove that one cannot identify the experimental tetanus with the voluntary or reflex one Consequently Cooper and Adrian's objections are not well founded

(4) *The functional synchronism of the nervous and muscular elements* In order to explain the differences of amplitude in the voluntary and reflex electromyogram Cooper and Adrian produce the following hypothesis "The explanation we take to be that the large waves represent the simultaneous discharge of impulses from the majority of the nerve cells and consequent contraction of the majority of the muscle fibres and that the small waves represent the discharge from a small number of nerve cells out of phase with the rest" It is therefore the theory which Gotch(9) put forward for the nerves and which these authors apply to the nervous centres But if the neurones can act individually or associate into a larger number when commanding a higher effort, it is not the same for the muscle fibres I have explained in my second memoir (pp 21, 22) why the "all or nothing" rule cannot be applied to the muscle of the skeleton and why the muscle fibres cannot act separately or by small groups as Keith Lucas(10) and Pratt(11) have maintained One of the principal reasons against that conception is the structure of the muscle Working with Dragoiu I have proved(12) the existence of a very abundant and very tight connective tissue between the fibres of the skeleton muscle by the aid of impregnation with reduced silver nitrate, according to Cajal's method

One can clearly see in these preparations both the envelope of every fibre, or sarcolemma which is of connective elastic nature, and the interstitial connective tissue, which binds the muscular fibres together very tightly In face of the fact that such is the structure of the striated muscle, Pratt's opinion, that the fibres of the muscle can freely slip over one another, becomes a material impossibility And Pratt relies in his experiments just on this slipping of the fibres, in order to prove that the

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For all these reasons, of structural and functional nature, I submit that all the fibres of the muscle contract at the same time when performing a certain work. The value of the work depends on the quantity of energy spent by each of these fibres and not on their number Thus the functional synchronism is an essential condition of the muscular work

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DESCRIPTION OF PLATES

PLATE I.

Figs 1 and 2 Voluntary electromyogram of the flexors of the fingers in man. String of silvered glass Diameter = 3μ. Resistance = 4000 ohms. Tension = 2 mm. per 0 001 v Magnification = 2000 times Time in fifths of a second.

Fig 3 Guinea pig Electroneurogram. Brain and sciatic nerve Voluntary walk. String of silvered glass. Diameter = 2 5μ. Resistance = 6500 ohms Tension = 2 mm. per 0 001 v Magnification = 1000 times Time in fifths of a second.

PLATE II.

Figs 1 and 2 Frog with brain destroyed Reflex electromyogram of gastrocnemius muscle. Temperature on the outside 27° String of silvered glass. Diameter = 2μ Resistance = 9000 ohms Tension = 0 8 mm. per 0 001 v Amplifier in the circuit Mechanical stimulation of fore limb Time in one hundredths of a second.

Fig 3 Frog with brain destroyed Total cooling Temperature in the œsophagus, 8 7° Reflex electromyogram of gastrocnemius muscle Same string as in Figs 1 and 2 Mechanical stimulation of fore limb Time in one hundredths of a second.

Fig 4 Frog with brain destroyed. Cooling of spinal cord. Temperature in the œsophagus = 9 5° Reflex electromyogram of gastrocnemius muscle Mechanical stimulation of fore limb Same string as in Figs 1 and 2. Time in one hundredths of a second.

500 per sec

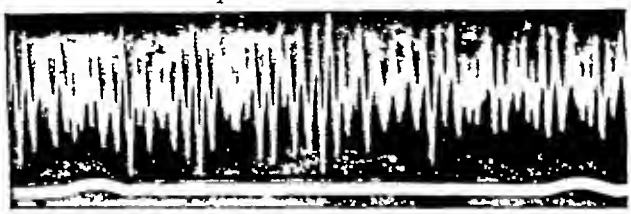

Fig 1

500 per sec

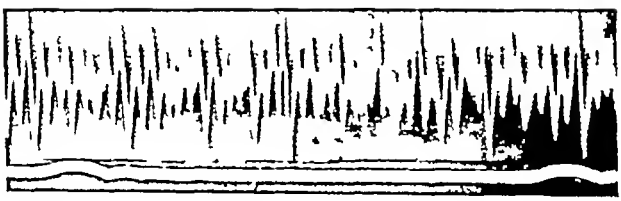

Fig 2

400 per sec Electro-cardiogram 400 per sec

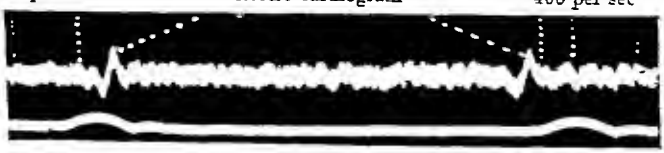

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PLATE II.

Figs 1 and 2 Frog with brain destroyed. Reflex electromyogram of gastrocnemius muscle. Temperature on the outside 27° String of silvered glass Diameter = 2μ Resistance = 9000 ohms Tension = 0 8 mm per 0 001 v Amplifier in the circuit. Mechanical stimulation of fore limb Time in one hundredths of a second.

Fig 3 Frog with brain destroyed Total cooling Temperature in the œsophagus, 8 7° Reflex electromyogram of gastrocnemius muscle Same string as in Figs. 1 and 2 Mechanical stimulation of fore limb Time in one hundredths of a second.

Fig 4 Frog with brain destroyed. Cooling of spinal cord Temperature in the œsophagus = 9 5° Reflex electromyogram of gastrocnemius muscle Mechanical stimulation of fore limb Same string as in Figs 1 and 2 Time in one hundredths of a second.

Fig 1

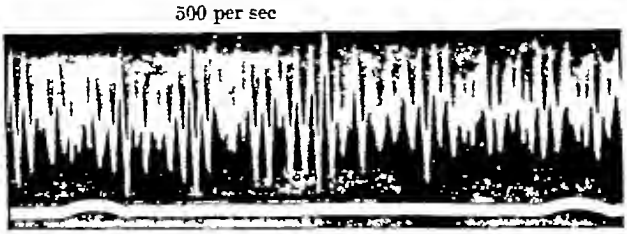


Fig 2

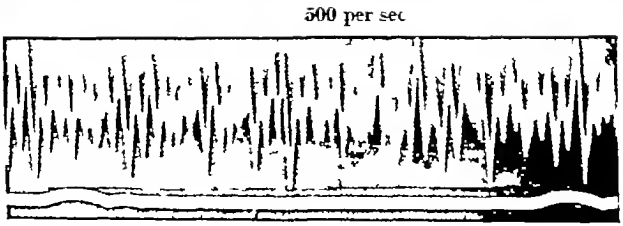
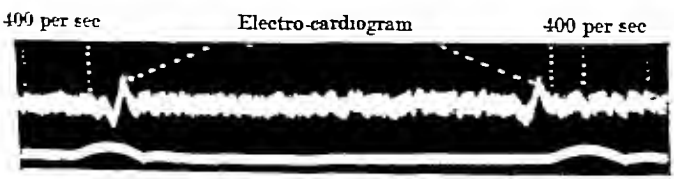


Fig 3



DESCRIPTION OF PLATES

Plate I.

Figs 1 and 2 Voluntary electromyogram of the flexors of the fingers in man String of silvered glass Diameter = 3μ. Resistance = 4000 ohms. Tension = 2 mm. per 0 001 v Magnification = 2000 times Time in fifths of a second

Fig 3 Guinea pig Electroneurogram Brain and sciatic nerve Voluntary walk. String of silvered glass. Diameter = 2 5μ. Resistance = 6500 ohms Tension = 2 mm. per 0 001 v Magnification = 1000 times Time in fifths of a second.

Plate II

Figs 1 and 2 Frog with brain destroyed Reflex electromyogram of gastrocnemius muscle. Temperature on the ontside 27° String of silvered glass Diameter = 2μ. Resistance = 9000 ohms Tension = 0 8 mm per 0 001 v Amplifier in the circuit. Mechanical stimulation of fore limb Time in one hundredths of a second.

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Fig 4. Frog with brain destroyed Cooling of spinal cord Temperature in the œsophagus = 9 5° Reflex electromyogram of gastrocnemius muscle Mechanical stimulation of fore limb Same string as in Figs 1 and 2 Time in one hundredths of a second.

COMPARATIVE EFFECT OF VARIOUS DRUGS UPON THE CORONARY CIRCULATION.

By G V ANREP and R S STACEY (*Scholar of Trinity College Cambridge*)

(*From the Physiological Laboratory Cambridge*)

In a series of recent papers[1 2 3] a method has been developed for registering the outflow of blood from the drained coronary sinus during a single cardiac cycle by means of the hot-wire anemometer The records already obtained by this method show three characteristic waves of increase in outflow and the relations of these waves to other events of the cardiac cycle have been worked out The first wave is caused by and follows the contraction of the auricle, the second and smallest is related to the isometric period of ventricular systole, while the third and largest wave is determined by the contraction of the heart during the ejection phase It had further been noticed that these three waves, which occur under widely different conditions of the heart beat may have a very different form. In some experiments they are sharply defined in others they merge one into another up to a point of complete fusion. When one or the other type of the coronary outflow was observed it would generally persist during an experiment, except in prolonged experiments in which the separate waves show a tendency to fuse together The difference between the various types of the coronary outflow is independent of changes in such circulatory conditions as arterial blood-pressure, cardiac output and heart rate

The question remains as to whether the differences in the coronary outflow curves depend on the strength of the cardiac contraction and it was the object of the experiments described below to obtain information on this point The changes in the strength of contraction were produced by administration of certain drugs whose effect upon the heart and upon the coronary circulation is already known

The experiments were performed on dogs, using the heart-lung preparation The coronary blood flow was recorded by the method described by Anrep, Cruickshank, Downing and Subba Rau[1] The heart rate was controlled in all experiments by stimulating the right auricle

fused with one another This change in form of the curves persisted after adjustment of the aortic pressure (Fig 2) The position of the

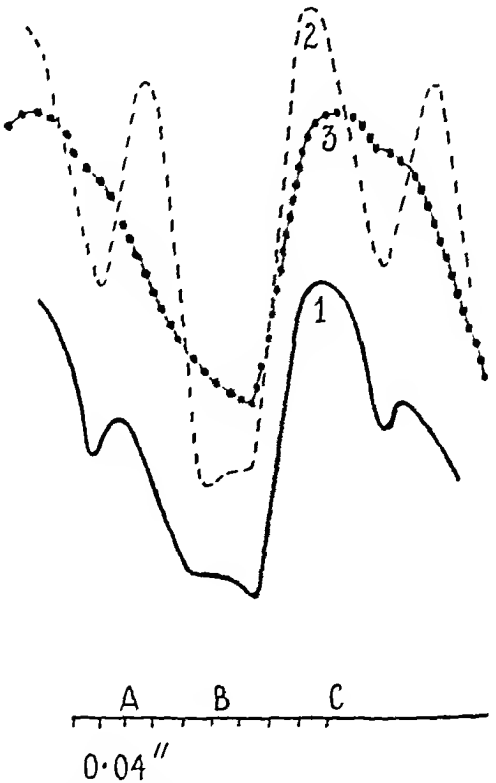

Fig 1 Redrawn, superimposed and corrected curves of coronary outflow recorded by the hot-wire anemometer Mean arterial blood pressure 95 mm Hg, systemic output is maintained at 650 c c and the heart rate at 150 beats per minute 1, normal curve, coronary blood flow—0 45 c c per beat, 2, effect of adrenaline (0 01 mgm)—0 73 c c , 3, effect of carbon dioxide (7 3 p c)—0 65 c.c , *A*, first or auricular wave, *B*, second wave related to the isometric period of ventricular contraction, *C*, third wave occurring during the ejection period

waves in the cycle was not appreciably altered except that the interval between the auricular wave and the first ventricular wave was shortened after adrenaline and lengthened after carbon dioxide, a change which is probably determined by the effect of these drugs upon the A.-V conduction[1] It is known that the strength of the heart beat is affected by

[1] The position of the waves in the cycle was determined in experiments in which the coronary outflow was registered together with the intra ventricular and aortic pressure

The drugs used were adrenaline, carbon dioxide, pituitary extract and caffeine These drugs have a definite effect upon the coronary circulation as well as upon the heart muscle It is, therefore, impossible, without further analysis to decide whether a change in the general appearance of the coronary outflow curve which follows the administration of any of these drugs is due to a decrease or increase in the coronary blood flow or to changes in the contraction of the heart In order to distinguish between these factors we examined the effect of the drugs, first allowing them to exert their full effect upon the heart and upon the coronary blood flow, and then under conditions in which the coronary blood flow was kept constant by adjusting the aortic blood-pressure We could thus compare the effect of the drugs at constant aortic pressures and at constant coronary blood flows, as will be made clear from the description below

The effect of adrenaline and carbon dioxide Adrenaline diminishes the volume of the heart and increases the strength of its contraction, carbon dioxide acts in the opposite manner, both, however, increase the coronary circulation. After a number of records of the normal coronary outflow had been obtained adrenaline or carbon dioxide was administered and a second set of records was taken The latter records showed the effect of the drug on the calibre of the coronary vessels themselves, they were therefore not suitable for comparison with the normal records In order to allow a more direct comparison the aortic blood-pressure was lowered to a point at which the coronary blood flow became equal to that observed before the administration of the drug A third set of records was then taken

In some experiments the effect of the drug was allowed to wear off, after which we increased the blood-pressure up to a point at which the coronary blood flow became equal to that observed during the effect of the drug, a fourth set of records being taken

Fig 1 shows three redrawn superimposed curves taken from a typical experiment a normal curve, a curve after administration of adrenaline and a third curve after administration of carbon dioxide The three curves are taken at a constant mean aortic blood-pressure Fig 2 shows similar curves but after adjustment of the blood-pressure to a level at which the coronary blood flow was constant in the three cases

In this experiment adrenaline and carbon dioxide increased the coronary circulation to nearly the same extent (Fig 1), the individual waves of the coronary outflow became more distinct after adrenaline, while during administration of carbon dioxide they flattened out and

fused with one another This change in form of the curves persisted after adjustment of the aortic pressure (Fig 2) The position of the

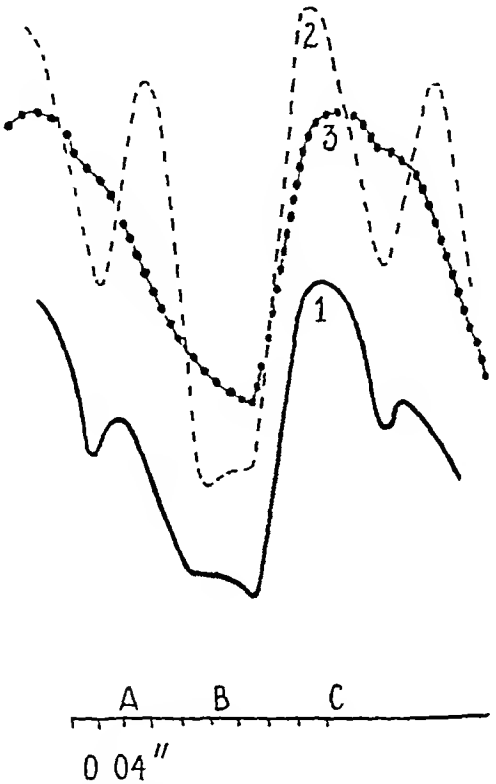

Fig 1 Redrawn superimposed and corrected curves of coronary outflow recorded by the hot wire anemometer Mean arterial blood pressure 95 mm. Hg systemic output is maintained at 650 c c. and the heart rate at 150 beats per minute 1 normal curve coronary blood flow—0·45 c.c. per beat, 2 effect of adrenaline (0·01 mgm.)—0 73 c c 3 effect of carbon dioxide (7 3 p c.)—0 65 c.c. *A*, first or auricular wave, *B* second wave related to the isometric period of ventricular contraction, *C*, third wave occurring during the ejection period.

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[1] The position of the waves in the cycle was determined in experiments in which the coronary outflow was registered together with the intra ventricular and aortic pressure.

considerably smaller concentrations of adrenaline or carbon dioxide than is the coronary blood flow Administration of either of the drugs in

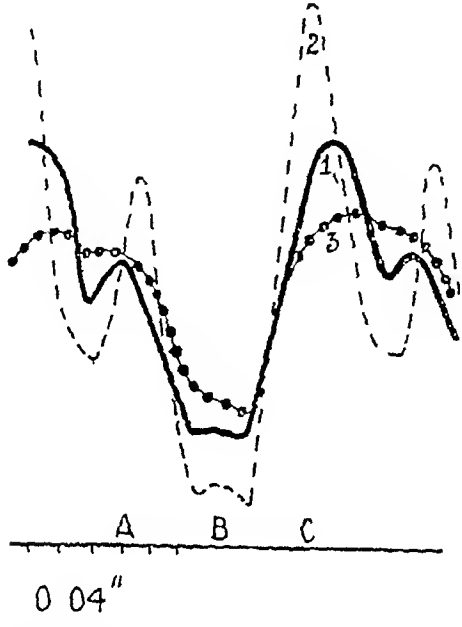

Fig 2 Same experiment as in Fig 1, but after adjustment of the aortic blood pressure so as to maintain the coronary blood flow constant 1 normal curve, aortic pressure 95 mm , coronary flow 0 45 c c per beat, 2 effect of adrenaline aortic pressure 78 mm , coronary flow 0 45 c.c 3 effect of carbon dioxide, aortic pressure 82 mm , coronary flow 0 46 c c

such small concentrations has a similar but less conspicuous effect upon the form of the coronary outflow curve to administration of the higher concentrations In these cases an adjustment of the aortic pressure is not, however, required since the amount of the coronary blood flow remains unaffected by the drug

The effect of pituitary extract and caffeine Pituitary extract and caffeine in the doses which were used had only a slight effect upon the heart muscle as measured by its diastolic volume Pituitary extract, however, produced a conspicuous diminution and caffeine an increase in the coronary circulation After readjusting the blood-pressure so as to maintain the coronary circulation constant we found the curves to be nearly superimposable on the normal curves except that after the

pituitary extract the rate of rise of the main wave became somewhat slower while after caffeine it became steeper

Summarising the results of these experiments it can be said that with none of the four drugs used is there any relation between their effects upon the heart muscle and upon the amount of the coronary blood flow The following table shows that the changes in the heart volume (which at a constant heart rate resistance and output is a measure of the strength of contraction (4)) and the changes in the coronary blood flow are independent of one another The data given in the table are taken from an experiment in which the drugs were administered in the order shown in the table the blood used in the preparation was replaced by fresh blood, after administration of adrenaline and pituitary extract, and time was allowed for the heart volume and the coronary blood flow to return to normal The weight of the heart in this experiment was 62 grm and the amount of blood in circulation was about 650 c c

The total output and heart rate were maintained throughout the experiment at 800 c c and 156 beats per minute respectively

	Change in coronary blood flow in p c	Change in heart volume before and after adjustment of the blood pressure in c c.		Change in mean aortic blood pressure required for adjustment, in mm. Hg
		Before	After	
Adrenaline 0·01 mg	− 85	−8 4	−9 5	From 100 to 80
Carbon dioxide 7 p c.	− 70	−7 5	−4 3	100 „ 87
Pituitary extract 0 1 c c (B D H.)	− 75	−2 4	+7 8	100 „ 145
Caffeine sodium benzoate 0 1 grm.	−115	−1 6	−4 5	„ 100 „ 55

Although there is no relation between the effect of these drugs upon (*a*) the amount of coronary blood flow and (*b*) the strength of contraction, the latter determines the character of the outflow curves When the heart beat is strengthened by administration of adrenaline the waves of the coronary outflow are sharply defined, whereas when it is weakened by carbon dioxide the separate waves fuse together The effect of pituitary extract and caffeine in the doses used is in this respect negligible But larger doses of pituitary extract have an effect similar to that of carbon dioxide, while large doses of caffeine produce changes similar to adrenaline

SUMMARY

From the study of the effects on the heart of adrenaline, carbon dioxide, pituitary extract and caffeine it is concluded that the effect of these drugs upon the strength of heart beat as measured by the heart volume bears no relation to their effect upon the amount of coronary blood flow But the form of the coronary outflow curves is dependent on the strength of the cardiac contraction, the three characteristic waves being accentuated during the increased strength of contraction produced by adrenaline and obliterated during the weakened contraction as a result of administration of carbon dioxide Small doses of pituitary extract and caffeine produce a negligible effect on both the strength of contraction and the form of the coronary outflow curve

The expenses of this research were defrayed from a grant by the Medical Research Council held by one of us (G V A)

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IS THERE "TRANSITIONAL DECREMENT" IN NARCOTISED NERVE?

By G KATO and D TERUUCHI

(From the Physiological Institute, Keio University, Tokyo)

In 1923 one of the present writers(1) made the first report on the decrementless conduction in a narcotised region of nerve Two kinds of results were obtained on this problem by experiments in a narcotising chamber They are as follow

A The time required for suspension of conduction from an outside electrode is not dependent upon the length of narcotised nerve, if the narcotised region is longer than 6 mm

B On the other hand, the time is dependent upon the length of narcotised nerve, if the narcotised region is shorter than 6 mm ("limit-length") in other words, below the "limit-length," the shorter the narcotised region, the longer time is required to suspend conduction

These two facts were demonstrated at the XIIth International Physiological Congress at Stockholm

The result A indicates that there is non-decremental conduction in a narcotised region of nerve under the stated condition And as to the result B we offered the following explanation The whole nerve within the narcotising chamber is in the same condition, and therefore, theoretically speaking, the depth of narcosis ought to be uniform over the entire length of nerve within the chamber But this is not the case in reality as it will be seen in Fig 1 The parts of nerve near the edge of the narcotising chamber receive the influence from the normal part outside, for instance by the diffusion of the narcotic out of the chamber along the fibres and by the inward diffusion of the normal tissue fluid or of Ringer's solution along the fibres from outside, giving a gradient of concentration of narcotic so that full depth of narcosis is only reached at a distance of about 3 mm from the chamber wall. The gradient of narcosis thus made may be represented diagrammatically by Fig 1 The Fig 2 shows the case in which the "limit-length" is narcotised If, therefore, the stretch of nerve to be narcotised is sufficiently short, no part of the nerve within the narcotising chamber can be free from the influence from the normal parts above and below as it is shown in

Fig 3, and the narcosis would proceed only to the point P at the stage of narcosis at which it would have proceeded to F in a longer region

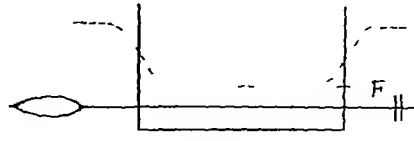

Fig 1

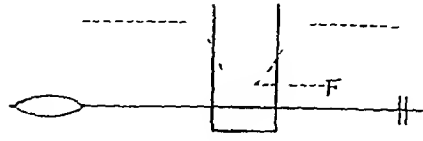

Fig 2

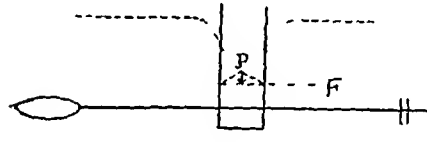

Fig 3

Thus the narcosis proceeds always slower in such a short region than in a longer one If we assume that the depth F of narcosis is needed for abolition of conduction from the outside electrode, then a longer time is required for it in a shorter region, because the narcosis has to proceed from P to F The "limit-length" is about 6 mm in the case of 2 0–3 0 urethane or cocaine solution

This point has, two years later, received an experimental proof by T Hayashi(2) who has determined the excitability of nerve within and without the narcotising chamber by means of a sharply localised mechanical stimulus

Previous to Hayashi's experiment, A Forbes and his collaborators(3), who also have proved the non-decremental conduction in a narcotised region with mammalian nerve, suggested the alternative possibility of "transitional decrement," saying that on passing from normal to narcotised nerve the impulse does not fall immediately to the lower level characteristic of narcosis, but is conducted with a decrement for a short distance before reaching its new level, although the local response at every individual point on the narcotised nerve is strictly all or none This suggestion seems apparently to agree with the results of Drury(4) and of Cattell and Edwards(5)

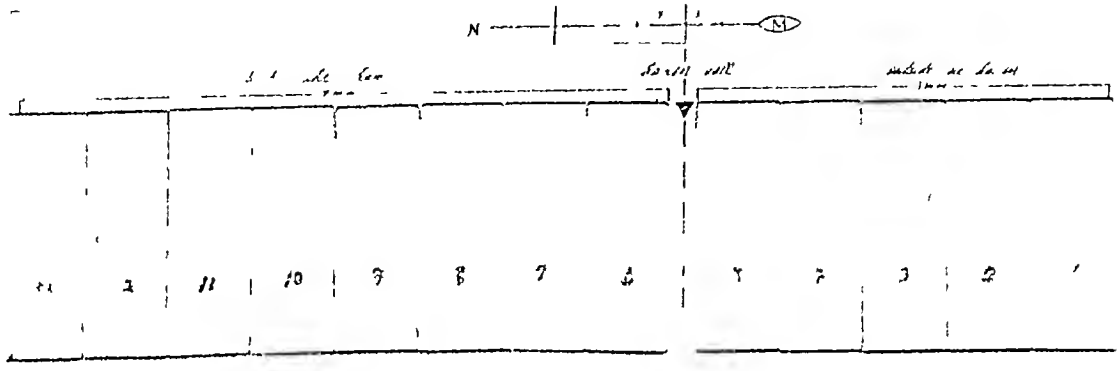

Fig 1

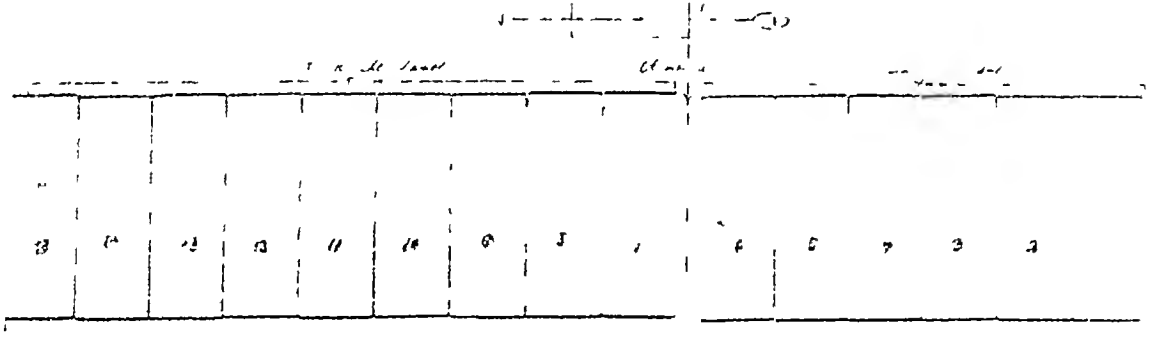

Fig 6

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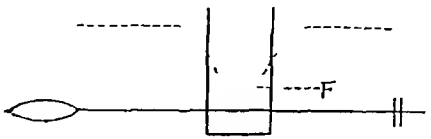
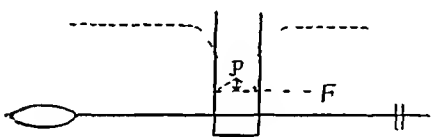


Fig 2



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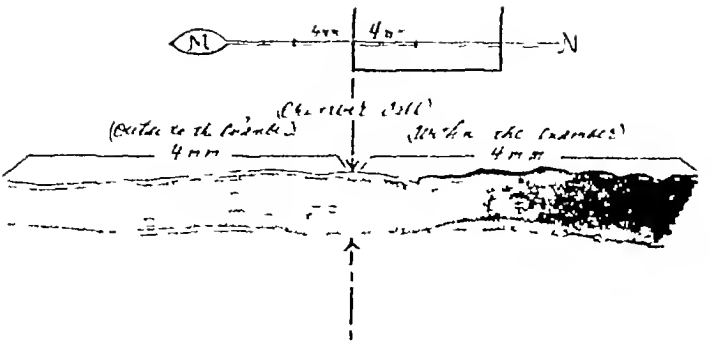

Fig 6

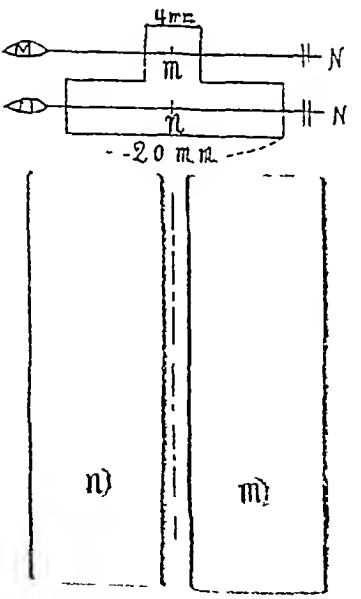

Fig 7

Whether the result B is due to the diffusion factor or to "transitional decrement" is a problem of much interest, although Hayashi's results offer important evidence in favour of the diffusion factor To answer this question we have chosen dye solution and used it with or without narcotic instead of using a simple narcotising solution alone, because by this means we can judge the distribution of the dye within the nerve by the depth of its staining

Exp 1 The nerve muscle preparation *NM* was drawn through the narcotising chamber in the usual way as we do in narcotising chamber experiment (see Fig 4) The chamber (with 1 mm thick wall) was then filled with 2 0–2 5 p c urethane-Ringer's solution which contains 1 0 p c methylene blue or 2 0 p c carmine After the conduction failed from the outside electrode, the part of nerve near the edge of the narcotising chamber was cut in serial transverse (or longitudinal) sections, each piece being 15–18 microns thick One example in which carmine was used as a dye is given in Fig 4 It will be seen that, on one hand, the dye diffuses out of the chamber more than 3 mm and on the other hand, the nerve inside the chamber is not stained in equal depth The colour becomes gradually deeper as the distance from the chamber wall increases until it reaches a full depth at about 3 5 mm from the chamber wall From numerous experiments made in this way with various concentration of the dye, it was found that the distance within which the influence from the normal part (*i e* gradient of narcosis) can be detected in the chamber becomes gradually greater as the concentration of the dye is decreased

Another example is given in Fig 5 in which methylene blue is used as a dye Fig 6 shows a longitudinal section of the nerve at the edge of the narcotising chamber We see the similar diffusion phenomenon here as in the transverse section

Exp 2 Two nerve muscle preparations taken from the same toad were passed through the same narcotising chamber such as shown in Fig 7, so that one nerve was exposed to the narcotising solution (containing dye) for a length of 20 mm , and the other for a length of only 4 mm (below the "limit-length") At the stage of narcosis at which the conduction failed in the longer region—the conduction does not yet fail in the shorter region, because it is below the "limit-length"—the two middle parts (*m* and *n*) of the both narcotised regions were taken out of the narcotising solution and were examined in serial transverse sections It will be seen in Fig 7 that the middle part *m* in the shorter stretch is not so deeply stained as the middle part *n* in the longer

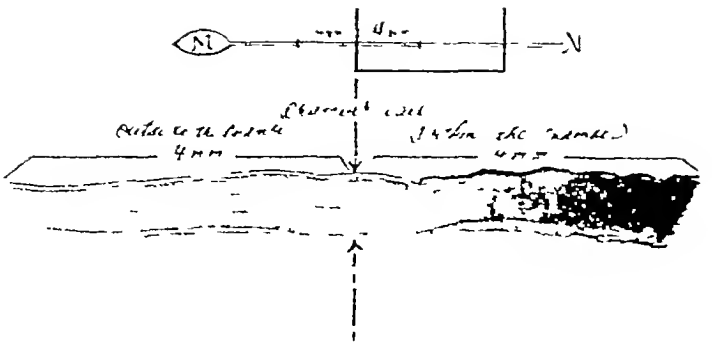

Fig 6

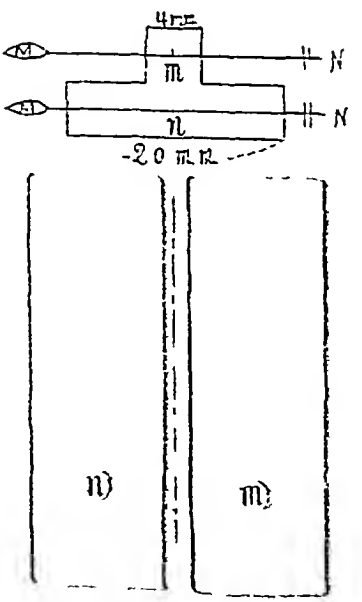

Fig 7

stretch This result is in complete agreement with what we have expected from Figs 1 and 3

From these results it can safely be concluded that the qualitatively same phenomenon occurs as to the diffusion of narcotic, although it may differ quantitatively Moreover, H Tamura made an interesting observation that the "limit-length" is different according to the kind of narcotic used and that it increases as the concentration of narcotic decreases, for instance in case of the chloretone

0 5 p c	4 mm	0 2 p c	8– 9 mm
0 3 p c	6 mm	0 12 p c	10–11 mm

In the case of abolition of conduction in isotonic sugar solution (without salt), the "limit-length" is not less than 15 mm

From these facts and also from the experimental results described elsewhere(2), we conclude that the result B in question can easily be explained by the diffusion factor alone, without assuming "transitional decrement"

SUMMARY

The experiments in the narcotising chamber show two kinds of results

A The time required for suspension of conduction is not dependent upon the length of narcotised nerve, if its length is longer than 6 mm

B The time is dependent upon the length of narcotised nerve, if its length is shorter than 6 mm

The result A represents the non-decremental conduction in narcotised region And as to the result B two kinds of explanation are suggested, a diffusion factor and a "transitional decrement" To determine this alternative a dye solution was used instead of using a simple narcotising solution, and the part of nerve near the edge of the narcotising chamber was examined in serial transverse or longitudinal section From the microscopical preparations thus made it was concluded that the result B can easily be explained by the diffusion factor alone, without assuming "transitional decrement"

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

October 15, 1927

The concentration of CO_2 in successive portions of an expired breath

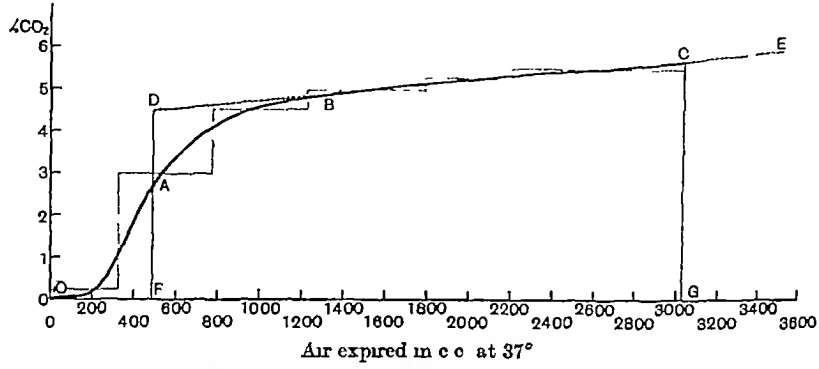
By R S Aitken and A E Clark-Kennedy

(*Medical Unit, The London Hospital*)

At its December meeting last year we demonstrated to the Society an apparatus which was designed to divide up a single expired breath into six successive portions The breath is taken during the tenth minute of moderate work on a bicycle ergometer, and varies from 2 to 3½ litres in size The six portions are measured and analysed for oxygen and CO_2, and their CO_2 concentrations are plotted against their volume, a smooth curve is drawn through the rectangular steps representing the successive portions, as shown in the figure

A considerable number of experiments of this kind has been done It is found that the experimental curve has a typical form an S-shaped rise from the origin up to 1000 or 1200 c c , followed by a straight portion sloping gently upwards The S-shaped portion represents alveolar air diluted with fresh air from the dead space (instrumental plus physiological), the straight portion represents undiluted alveolar air In this type of breathing, then, the alveolar CO_2 concentration during more than half the expiratory phase is rising at a steady rate It seems fair to assume that during the earlier part of expiration it also rises at the same rate, in that case it can be represented by a backward prolongation of the straight portion of the experimental curve, *i e* by the dotted line *BD* in the figure If the length *BD* be such that the area *FDCG* equals the area *OABCG* (each area representing the amount of CO_2 in the whole breath), then *D* indicates the alveolar CO_2 concentration at the beginning of expiration Moreover, the distance *OF* represents the volume of the total dead space In the same fashion, the straight portion of the experimental curve can be prolonged to the right to *E* over a distance equal to *OF*, to represent the CO_2 concentration of the air left in the dead space at the end of expiration *DE* then represents the changing alveolar CO_2 concentration throughout this expiration, in the breaths we have investigated the rise is about 0·5 p c CO_2 per litre of air expired

The mid-point of *DE* is the average CO_2 concentration during expiration In a 3-litre breath, it is about 0 5 p c lower than the CO_2 concentration of the last 10 c c of the breath, which are regarded by Yandell Henderson(1) as average alveolar air, and are collected by his automatic sampler and by Clark-Kennedy and Owen's(2) modification of it



The concentration of CO_2 in successive portions of an expired breath
OABC Experimental curve
DE Deduced curve representing alveolar CO_2 during expiration
Duration of expiration 1 77 secs
Taken during work CO_2 output 2100 c c per min. at 37°

The physiological dead spaces obtained by the above method lie between 280 c c and 392 c c, and in nine experiments on one subject they show a definite relationship to the volume of the tidal air (in the last minute of the experiment), being greater when the breathing is deeper, as shown in this table

Exp No.	Average Tidal Air c.c. at 37°	Physiological Dead Space c.c. at 37°
18	1890	283
20	1930	290
19	1940	280
23	2356	287
22	2371	295
24	2760	327
21	2880	361
26	3410	378
25	3520	392

These values for the dead space are somewhat lower than those which were found by Douglas and Haldane(3) during exercise, they are considerably higher than those of Krogh and Lindhard(4)

1 Henderson and Haggard. Amer Journ. Phys. 73 p 193 1925
2 Clark Kennedy and Owen. Quart Journ Med 20 p 383 1927
3 Douglas and Haldane This Journ. 45 p 235 1912
4 Krogh and Lindhard. This Journ. 47 p 30 1913–14

Observations upon the respiratory exchange, temperature and sugar in the blood of anæsthetised animals By E T Conybeare, H. B A. R Densham, M Maizels and M S Pembrey

A series of observations has been made upon rabbits before, during and after anæsthesia produced by urethane or ethylene

Sugar in the blood The values are given in mgm per 100 c c , the blood was drawn from the auricular vein.

		mgm of sugar per 100 c.c. of blood	No of observations	No of animals	
Before anæsthesia	Fasting	105–152	9	9	
,,	Fed	105–200	18	18	(M)
,,	,,	96–200	40	7	(D)

During anæsthesia produced by urethane a rise in the sugar of the blood was observed in each case, and, as a rule, was as great in the fasting rabbits as that observed in the well-fed animals The increase was greater when the anæsthesia was deep

Urethane anæsthesia	Range of increase in sugar in blood		
	1st hour	3rd hour	
Light	9–49	25–130	Fasting
,,	25–38	67–153	Fed
Deep	48–169	100–290	Fasting
,,	42–166	99–259	Fed

The rectal temperature fell during anæsthesia, the lowest record was 26 8° in a rabbit with the sugar of the blood raised to 456 after 24 hours' anæsthesia As a rule the lowest temperatures were associated with the highest values for the sugar in the blood and the lowest values for the respiratory exchange

Ethylene Anæsthesia

Animal No	Sugar in blood before anæsthesia	Average sugar in blood during anæsthesia	Average rectal temperature during anæsthesia	Tolerance to sugar during anæsthesia	Average rectal temperature at the time of the test for tolerance
VIII	150	152	37 5°	Decreased	34°
		305	33 4°		
IX	125	100	36 5°	Decreased	34 5°
		255	32·0°		
X	120	130	37 2°	Increased	36 5°
			35 5°	Decreased	34 6°
XIII	122	113	36 5°		
XIV	100	80	37 5°		
XII	170	157	37 0°		

When the rabbit remained under the influence of urethane the sugar in the blood was maintained at a high level during the second and third

days, even in the case of animals which had fasted before the injection of the drug

Condition	Maximum of sugar in the blood 1st day	2nd day	3rd day	Anæsthesia
Well fed	343	284	—	Deep
,,	426	260	—	,,
,,	316	180	110	,,
,,	340	290	176	Light
,,	318	230	205	Deep
,,	256	348	—	,,
Fasting	444	430	—	,,
,,	313	436	260	,,
,,	442	366	—	,,
,,	262	399	—	Light
,,	256	440	—	,,
,,	304	340	—	Deep
,,	260	250	—	Light
,,	323	294	—	,,
,,	420	340	330	Deep

A few observations have been made on the sugar passed in the urine and the amount of glycogen in the liver under different conditions of anæsthesia One rabbit weighing 2 2 kgm excreted 3 3 grm of sugar in 48 hours of anæsthesia, another 3 5 grm in 36 hours

A large number of observations upon the influence of anæsthetics on the respiratory exchange has been made, there are special difficulties with ether and ethylene The following is an example of the effects of urethane, the periods are for 1 hour

Time	H_2O grm.	CO_2 grm.	O_2 grm.	$\frac{CO_2}{O_2}$	Rectal tem perature	Sugar in blood mgm in 100 c c.	Remarks
	1 60	2 66	2 08	0 93	38 5°	131	Before injection, well fed rabbit, weight 1 7 kilo
After 4 hours under drug	0 79	2 24	1 77	0 91	35	230	Deep anæsthesia
23 hours under drug	0 73	1 13	1 28	0 65	35	256 300	,,
28 hours under drug	1 06	1 42	1 35	0 76	31	348	,

There appears to be evidence of the production of sugar from fat

The difference of *p*H between plasma and red cells

By A C Hampson and M Maizels

Experiments were undertaken to determine the *p*H of the plasma and of the red cells in normal subjects and in certain cases of anæmia

Blood was drawn from a vein and allowed to run under paraffin in a centrifuge tube Heparin was used as an anti-coagulant The blood

was centrifuged and the *p*H of the plasma was determined at room temperature by means of the glass electrode The cells were laked by freezing and brought to room temperature The *p*H was then determined

In normal individuals the following results were obtained

TABLE I. ($T=20°$ C)

	*p*H of plasma	*p*H of cells	Difference
1	7 485	7 382	0 103
2	7 490	7 398	0 092
3	7 530	7 421	0 109
4	7 535	7 421	0 114
5	7 490	7 385	0 105
6	7 500	7 415	0 085
7	7·490	7·420	0·070
8	7 472	7 421	0·051
9	7 422	7 330	0 092
10	7 490	7 370	0 120
Mean	7 490	7 396	0·094

In patients suffering from pernicious anæmia similar estimations were made and in addition, the percentage of hæmoglobin in the blood and the amount of bilirubin in the plasma was determined (Table II)

TABLE II. ($T=20°$ C)

	*p*H of plasma	*p*H of cells	Difference	p c. Hb	v d. Bergh's test
1	7 665	7 182	0·483	20	+ + +
2	7 485	7 082	0·403	50	+ +
3	7 505	7 228	0 277	20	+(+)
4	7 450	7 221	0·239	80	+
5	7 490	7 288	0·202	90	(+)

Several cases of acholuric jaundice were also examined, the fragility to hypotonic saline being also determined —

TABLE III. ($T=20°$ C)

	*p*H plasma	*p*H cells	Difference	p c. Hb	v d. Bergh's test	Fragility to Hypotonic saline: Began p c.	Fragility to Hypotonic saline: Complete p c.
1	7·488	6 981	0·507	88	+ + +	0 72	0 45
2	7 420	7·012	0 408	52	+ +	0 78	0·45
3	7 472	7 120	0 352	65	+(+)	0 68	0·39
4	7 474	7 174	0 300	60	+	0 66	—

The *p*H readings were taken at room temperature, but, as C J Martin has shown, the correction for temperature is probably the same for cells and plasma The difference in *p*H between cells and plasma at room temperature may, therefore, be taken as being the same at body temperature

It will be noted that in normal subjects, the average difference of *p*H between cells and plasma was 0 094, the extremes being 0 051 and 0 120 The cells were more acid.

In five cases of pernicious anæmia, the *p*H difference varied between 0 20 and 0 48.

In four cases of acholuric jaundice, the *p*H difference varied between 0 30 and 0 50

A further case of acholuric jaundice, not included in Table III, was examined This case had been treated by splenectomy and had so improved as to appear almost normal The hæmoglobin was 98 p c , and the van den Bergh test was negative, although slight hæmolysis occurred in 0 60 p c saline The *p*H difference in this patient was 0 122—hardly above normal

In two cases of non-hæmolytic anæmia, the *p*H differences were 0 037 and 0 120 respectively

In the hæmolytic conditions, the difference of *p*H between cells and plasma was increased as compared with the normal this difference varying directly with the degree of hæmolysis (as estimated by the van den Bergh reaction for bilirubin), and not with the degree of anæmia, nor with the alteration of fragility of the red cells to hypotonic saline

In the hæmolytic conditions, the increased difference of *p*H was due to the red cells being more acid, the *p*H of the plasma being within normal limits—except in case 1 of Table II, where the patient was markedly dyspnœic and the plasma was more alkaline than normal

The effect of injections of pituitary extract, adrenalin and insulin on Ketonuria By J H Burn and H W Ling (*Preliminary communication*)

Rats, weighing from 100–150 grm , have been kept in metabolism cages of the Hopkins pattern, and fed on a diet of filtered butter Wigglesworth(1) showed that in this condition rats develop Ketonuria, which attains a maximum about the third day, and which for the most part disappears by the sixth day The acetone bodies have been estimated in the urine by the method of van Slyke(2)

We find that the Ketonuria produced varies in amount according to the time of year It is greatest in May and June, and least in winter Co ri and Cori(3) have observed a similar seasonal variation in the Ketonuria occurring in fasting rats

We have found that, in May and June, injections of pituitary extract (1 unit per rat given four times a day), on the second and following days of the fat diet, greatly diminish or inhibit the Ketonuria

Injections of adrenalin (0 04 mgm. per rat given four times a day) have the same action All experiments in which injections were made were controlled by observations on the same animals in a second period of fat feeding after an interval of three weeks It is not easy to demonstrate any inhibitory action of pituitary extract on the small Ketonuria which occurs in winter

We have some evidence, which is incomplete, that a single intravenous injection of 0 3 mgm thyroxine on the first day of the fat diet inhibits the Ketonuria also

In surprising contrast to these effects is the effect of injections of insulin Injections varying from 0 2 to 0 4 unit per rat have been given four times a day on the second and third days of the fat diet These injections have been enough to produce collapse though only rarely convulsions

As a result, the second day Ketonuria is almost always increased the increase being in the neighbourhood of threefold The third day Ketonuria is also usually increased but often it is less in amount than on the second day (whereas in the absence of injections a third day maximum is the rule) When on the fourth day no insulin is given, the Ketonuria usually diminishes at an abnormally rapid rate, and remains very small on the fifth day In the absence of more injections a second period of Ketonuria supervenes on the sixth and seventh days, disappearing again on the eighth day This second period can be suppressed by injections of adrenalin or pituitary extract, but not by injections of insulin The second Ketonuria is not observed in winter

(1) Wigglesworth. Biochem. Journ. 18 p. 1203 1924.
(2) van Slyke Journ. Biol. Chem. 32. 1917
(3) Cori and Cori. Journ. Biol. Chem. 72 p 615 1927

The compensation by the skin vessels during over-ventilation in man By R A. Collier, H. B A R Densham and H M Wells (*King's College, London*)

Dale and Evans found a considerable fall in systemic blood-pressure on over-ventilating anæsthetised cats Yandell Henderson has recorded a similar change in dogs, but in man he has found that there may be little or no change in the blood-pressure

These results having been confirmed, it seemed to be of interest to determine what factor is responsible for the maintenance of the blood-pressure in over-ventilation in man Yandell Henderson has shown that it is not the heart, so that it is presumably the blood vessels

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Dale and Evans showed that over-ventilation of an anæsthetised cat is associated with an increase in the volume of its limbs Nahun showed that there is a decrease in limb volume when a normal man over-ventilates, while Stewart found a decrease in the circulation through the hand, and Wells found a fall in the electrical resistance of the skin under the same circumstances

It appeared then that the vessels of the skin play some part in maintaining the blood-pressure during over-ventilation in man. This hypothesis seems largely confirmed by the fact that, in an individual whose blood-pressure normally rises on over-ventilation, there is a fall in blood-pressure when the skin vessels are inactivated by immersing him in a bath at either 15° C or 40–45° C

In the chloralosed cat compensation by the skin vessels appears to be absent, as judged by absence of a reduction of limb volume or of electrical resistance of the skin, but this is not due to a loss of excitability of the cutaneous vessels due to anæsthesia since they react to adrenaline, to alkali and, reflexly, to sensory stimulation, as shown by the same methods

In such an animal there must be, therefore, an absence of some stimulation which excites cutaneous vaso-constriction in man during over-ventilation

The only possible stimuli not present in the chloralosed cat are those concerned with the active forced respirations, which it is suggested bring about constriction of the vessels of the skin in man during over-ventilation and maintain the blood-pressure

The influence of ether anæsthesia upon the gaseous composition of blood By N E Pitt (*Preliminary Note*)

The oxygen of arterial blood is found to be lowered about two volumes per cent There is a rapid fall in the O_2 as soon as anæsthesia is induced and during the state there is some variation according to the depth of anæsthesia—when the rabbit is more deeply under the anæsthetic the O_2 falls still lower

Exp 10

	Vols per cent.		
	CO_2	O_2	N_2
Normal animal	47·0	16 6	1 6
Light anæsthesia	39 3	15 0	1 4
	42 2	14 9	1 6
Deep anæsthesia	44 2	14 2	1 5
	42 0	15 0	1 8
Recovery	47 9	16 1	1 4

The fall of O_2 content cannot be removed entirely when 60 p c of O_2 is given with the inspired vapour, but with 80 p c O_2 it entirely disappears

Ether anæsthesia (after the effect of induction has passed off) causes a lowering of the CO_2 value, usually to the extent of 5 to 10 vols per cent, and the fall varies inversely with the depth of anæsthesia, *i e* light anæsthesia is marked by a low CO_2 value and deep anæsthesia involves a high CO_2 In deep anæsthesia the CO_2 value may exceed the normal

The high CO_2 values found by previous observers in cases of chloroform anæsthesia correspond to the deeper stages of ether anæsthesia

The extent of the lowering of O_2 content is too great to be explained entirely as a result of shallow respiration, more especially when the occurrence of such low parallel CO_2 readings is taken into account In view of this the rate of gas exchange of normal and anæsthetised blood was compared (A constant volume of air was bubbled through a constant quantity of blood in 30 seconds and the blood gases were measured before and after) These experiments demonstrated a decreased rate of absorption of O_2 (about one-third normal) and an increased rate of loss of CO_2 (about three times normal) in the anæsthetised blood

Exp 15

	Vols of gas per cent. in blood						O_2 gain	CO_2 loss
	CO_2	O_2	N_2	CO_2	O_2	N_2		
Normal	52 0	14 7	1 6 →	50 3	16 8	1 7	= 2 1	1 7
Etherised	41 0	14 5	1 6 →	34 4	15 2	1 6	= 0 7	6 6

Comparing plasma and blood, the rate of CO_2 loss is much greater in plasma, *i e* the cells normally exert a hindering effect on excessive CO_2 loss Thus the effect of ether on this rate of CO_2 loss can be classified under the heading of "decreased activity of the cell"

Exp 19

Normal blood lost 1 5 vols. per cent. CO_2 by air treatment
Its plasma lost 5 5 vols per cent CO_2 by air treatment

The preparation of oxyhæmoglobin crystals from ox blood

By Wilfrid Marshall

A reliable method of preparing oxyhæmoglobin crystals in quantity from ox blood has not been described Hoppe-Seyler's method is tedious and with slaughter-house blood, except that of the pig, is not satisfactory Most of the irksome manipulations are unnecessary It is desirable to work at as low a temperature as is convenient, but the

preliminary work may be carried out at room temperatures, the ether may be advantageously used in place of part of the alcohol to aid crystallisation, and the stay in the separator need not be unduly prolonged The most objectionable feature of Hoppe-Seyler's method, however, is the use of undiluted alcohol When added to an ox blood solution, however slowly, to one-third the volume recommended, it causes considerable decomposition of the hæmoglobin The use of diluted alcohol greatly diminishes this change The following method was found to give, on the whole, the best results

Simplified Method Centrifuge the ox blood Remove the serum and wash the residual corpuscles three times with 1 p c salt solution Measure the separated corpuscular mass and add an equal quantity of tap water, shake well Place the mixture in a separator, add an excess of pure ether, shake and allow to stand overnight for separation to occur Run off the bottom clear solution, add to this by rapid dropping during constant agitation, half its volume of a mixture of 70 parts commercial alcohol (96 p c) and 30 parts ether-saturated water Allow to stand half an hour, filter Put the filtrate into a refrigerator or vacuum-freezer at − 5° C Crystals separate within 24 hours and a large crop is obtained in 2–3 days

If time is pressing, the lower part of the blood mixture may be run off after about 2 hours' stay in the separator and filtered, and the diluted alcohol then added The maximum concentration of alcohol which the mixture will tolerate without apparent change is 16 p c This strength, obtained by running slowly into the blood solution half its volume of 50 p c alcohol in ether-saturated water, however, gives only a small yield of crystals and occasionally produces slight turbidity on standing which interferes with crystallisation.

A further crop of crystals can be obtained from mother liquors by adding slight excess of pure ether and returning to the refrigerator

The moist crystals at room temperatures, or slightly above, dissolve in little more than their own weight of water Recrystallisation is effected by pouring off the supernatant fluid, washing the crystals rapidly with ice-cold ether-saturated water, melting the crystalline mass by the warmth of the hands or of tepid water, filtering to remove any trace of amorphous material, and replacing in the refrigerator The second crop of crystals is collected on a porous tile and, after the excess of fluid has drained off, the crystals are dissolved in the smallest quantity of water and the solution, after filtering, is kept at a temperature of approximately 0° C Freezing of the liquid must be avoided

The crystals may be kept in a moist state in a closed vessel filled with

oxygen for considerable periods with but slight change to methæmoglobin If air-dried, the crystals are converted into methæmoglobin but retain their form Dried in a desiccator over sulphuric acid they are changed to a substance giving a brown solution with no characteristic absorption bands

Benzene, chloroform, methyl, propyl, or other alcohols as adjuvants to, or substitutes for, ethyl alcohol offer no advantages

Effect of drugs on protein content of cerebro-spinal fluids of rabbits By L F Hewitt and H Florey

In the course of some other investigations it became desirable to know whether the permeability to proteins of the cerebral blood vessels could be altered by the administration of drugs

The procedure adopted for the collection of the cerebro-spinal fluid was devised to avoid any possible blood contamination The rabbit was rapidly killed by chloroform and its head then fixed in a flexed position so as to put the posterior atlanto-occipital membrane on the stretch A longitudinal incision was made in the mid-line down to this latter structure and the muscles retracted so as to bare it The membrane was thoroughly dried with cotton-wool A small hole was then burnt through it by means of a red-hot needle Through this hole the fluid—quite clear and blood free—was sucked into a dry glass pipette This method of collection was found to yield about 1 c c of fluid

The following drugs were used in an endeavour to increase the protein content—*para*phenylenediamine, urotropine, pilocarpine and histamine

*Para*phenylenediamine gives a remarkable œdema of the head and neck which has been found to be due to the escape of whole plasma from the blood vessels concerned (Tainter and Hanzlik) It was thought that this drug might alter the cerebro-spinal capillaries in the same way

Urotropine passes into the cerebro-spinal fluid and it is claimed by de Arric and Millet, who used a precipitin technique, that the permeability of the capillaries is also increased slightly Pilocarpine is a "lymphogogue," and histamine increases the permeability of blood vessels in parts of the body

The volume of the fluid was measured into a small centrifuge tube, 0 4 c c of 10 p c sodium tungstate and 0 5 c c of 8 p c sulphuric acid were added The tube was centrifuged and the supernatant fluid decanted off The precipitate was suspended in water, dissolved by adding one drop

of 20 p c sodium carbonate solution, 0 05 c c of a phenol reagent (Wu) and 0 5 c c of 20 p c sodium carbonate were added and the volume made up to 5 c c The colour produced was compared with that given by tyrosine with the same reagents, whence the protein content may be calculated

Drug	Dose g per kg	Fluid taken after	No of animals	Protein p c.
None	—	—	4	0·040
p Phenylenediamine	0 2 subcutis	2½ hrs	2	0 052
Urotropine	1 0 intraven.	1½ hrs.	1	0 040
Histamine	.2 5 mg	30 mins	1	0 036
Pilocarpine	0 6 mg	2½ hrs	1	0 040

In no case was there an appreciable alteration in the protein content except in the case of *p*-phenylenediamine where a 25 p c increase was observed, but this apparent increase is negligible compared with that obtained in man in tubercular meningitis, *i e* usually about 400 p c *p*-Phenylenediamine evidently passed into the fluid since on standing a blue colour developed The figures for normal rabbits are higher than those for human fluids

Recovery of a pancreatic secretory excitant by vivi-dialysis of the circulating blood By H Necheles and R K S Lim

(Peking Union Medical College)

Following the fundamental discovery of *secretin* by Bayliss and Starling (1902), others, viz Enriquez and Hallion (1903), Fleig (1903, 1904), Matsuo (1912–3), have succeeded in demonstrating the existence of a pancreatic excitant in the circulating blood The final proof, however, *i e* the identification of the circulating excitant with secretin, is still lacking It is, of course, essential first to isolate the excitant from the blood We wish to report the results of preliminary experiments in which we have endeavoured to recover the excitant from the circulation by vivi-dialysis

Dogs were starved for 16–36 hours, the carotid-jugular or the femoral vessels were cannulated under local anæsthesia and connected with the dialysing cylinders[1] Heparin was given as anticoagulant After a control dialysis of 2 hours, the dog was fed or *N*/10 HCl was injected into the duodenum (in the latter case, a general anæsthetic was employed) The dialysate (Locke's solution) was then changed, and the experiment continued for another 2 hours The dialysates were used unconcentrated

[1] Details of technique described by H Necheles in *Chinese Journ Physiol* 1 169–80 1927

or after concentration and precipitation of most of the salts with alcohol The final substance, a yellow oily fluid, was tested for its pancreatic exciting property on normal dogs under ether

Eight experiments were performed Two of these were vitiated in that the blood pressure fell as the experiment proceeded, resulting in a slow circulation rate and some clotting in the dialysers In both experiments, the control dialysate gave a positive result, while the dialysate obtained after feeding was less or equally potent

The injection of the unconcentrated dialysate failed to show any pancreatic effect

The remaining five experiments were apparently carried out under favourable conditions Of these three (one *N*/10 HCl in duodenum) gave positive results, the control dialysate having little or no effect, while the dialysate after feeding produced a pancreatic response One (*N*/10 HCl in duodenum) was completely negative, while another gave equally positive responses to both dialysates

There is thus undoubtedly a pancreatic excitant in the circulation, which may be present before stimulation and which in three out of five satisfactory experiments was increased after stimulation We were not able to state the occurrence of an increase after stimulation in the case of the gastric excitant (Lim and Necheles, 1926)

Since both gastric and pancreatic excitants were prepared in the same way, the possibility of their identity is not remote The identity of our dialysed excitant with secretin obtained from intestinal extracts, remains to be investigated, although we have found that Luckhardt's method of salting out secretin from extracts, fails to precipitate the dialysed excitant

VOL LXIV No 2

CONTENTS

Notice to Contributors All papers should be directed to

THE EDITOR OF THE JOURNAL OF PHYSIOLOGY,
University College,
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and not to any other address

Papers sent for publication should be typed and the results given in as concise a form as possible Protocols should be illustrative only Figures should be ready for photographic reproduction Diagrams should be in Indian ink and plain white or faint blue lined paper only should be employed letters, numbers, etc, should be written *in pencil* Every paper must be accompanied by a summary not exceeding in length five per cent of the paper

Contributors of papers involving extensive numerical observations are requested to consult the recommendations of the British Association Committee on Biological Measurements, 1927, obtainable from the British Association, Burlington House, W 1 *Price 6d*

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